

PENETRATION OF ERYTHROMYCIN

TO THE MIDDLE EAR AND WALDEYER'S RING
AND ITS EFFECT ON THE
NASOPHARYNGEAL PATHOGENS

by
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In gratitude to my parents



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The present thesis is based upon the following publications, which will be referred to in the text by their Roman numerals.

- I. Sundberg, L., Edén, T., Ernstson, S. & Pahlitzsch, R.
Penetration of erythromycin through respiratory mucosa.
A study using secretory otitis media as a model.
Acta Otolaryngol (Stockh), Suppl. 365, 1979.
- II. Sundberg, L., Edén, T. & Ernstson, S.
Penetration of erythromycin in Waldeyer's ring -
adenoid tissue.
Acta Otolaryngol (Stockh), Suppl. 384, 1981.
- III. Sundberg, L., Edén, T. & Ernstson, S.
Penetration of erythromycin in Waldeyer's ring -
tonsil tissue.
Acta Otolaryngol (Stockh), Suppl. 384, 1981.
- IV. Sundberg, L., Cederberg, Å., Edén, T. & Ernstson, S.
Bacteriology in secretory otitis media.
Acta Otolaryngol (Stockh), Suppl. 384, 1981.
- V. Sundberg, L., Cederberg, Å., Edén, T. & Ernstson, S.
The effect of erythromycin on the nasopharyngeal
pathogens in children with secretory otitis media.
Acta Otolaryngol (Stockh) 1983. Accepted for
publication.

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Abbreviations:

AOM	acute otitis media
MEE	middle ear effusion
MIC	minimum inhibitory concentration
OME	otitis media with effusion
SOM	mucoid or serous otitis media

Definitions:

Otitis media with effusion (OME) has recently been proposed to replace old terms such as catarrhal otitis media, chronic serous otitis media, secretory otitis media, glue ear etc. (Paparella, 1976; Senturia et al., 1980). Depending on the nature of the middle ear effusion (MEE), OME has been divided into the following subgroups: *purulent otitis media*, *serous otitis media* and *mucoid otitis media*, all different manifestations of otitis media.

It should be borne in mind, that a correct judgement of a MEE behind an intact tympanic membrane might be difficult and sometimes impossible without a paracentesis (Axelsson et al., 1979). In the present investigation SOM is defined as a non-purulent effusion, either mucoid or serous, behind an intact drum.

INTRODUCTION

The serendipitous observation by Fleming (1929), that mould cultures inhibited the growth of adjacent bacteria marked the birth of a new era in antimicrobial therapy. The introduction of antibiotics in clinical practice fundamentally changed the panorama of infectious diseases. In the field of ENT, the previously life-threatening complications of AOM became rare, while less dramatic conditions like SOM appeared to increase in frequency (Lim et al., 1980, Qvarnberg, 1981). The present situation with a tendency to overuse or even abuse antibiotics, is, however, alarming, and it emphasizes the need of using antibiotics only on strict indications (Kallings, 1982).

Nevertheless, when using antibiotics whether on new or old indications, a prerequisite for successful therapy is to achieve sufficient concentrations *at the site of infection*. As early as 1913, Ehrlich postulated that the chemotherapeutic effect of a substance depended on the relationship between the lowest concentration, which, *in vitro*, inhibited the growth of bacteria, and the concentration, which was achieved in the host organism. In the complex interaction between the host's defence mechanisms (Naumann, 1980), the virulence of the invading bacteria and the properties of the drug, an adequate antibiotic concentration at the focus of infection may be decisive for the outcome of the disease by tipping the scale in favour of the host.

Knowledge of the antibiotic concentration in plasma or blood does not always give reliable information of the levels obtained in extravascular compartments. It is therefore necessary to study antibiotic penetration to tissues and secretions (Andersson et al., 1976; Barza, 1981, Ögren & Cars, 1982).

Different methods of following antibiotic penetration into interstitial fluids have been elaborated, e.g. the skin blister

technique, the tissue cage model and the cotton thread operated into tissue (Simon, 1976; Chisholm, 1978, Cars, 1981). In the commonly used tissue homogenization technique, the cell membranes are inevitably destroyed (Ögren & Cars, 1982). Therefore, tissue homogenates give data on the mean antibiotic concentration in both the extra- and intracellular compartments. The concentration of antibiotics that are mainly localized to the interstitial fluid will consequently be underestimated.

In respiratory tract infections, the bacterial colonization primarily takes place in the secretions covering the respiratory mucosa (Lundberg & Lönnroth, 1979). It is only, when the normal arrangements of the epithelial cells disrupt, that a bacterial invasion of the interstitial fluid may take place (Lundberg et al., 1981). Under these circumstances, it is important to study the antibiotic concentration in respiratory secretions, and, if possible, in patients with an inflamed respiratory mucosa and in the actual age group. The SOM model (see page 12) fulfils in many respect these requirements.

The middle ear and the nasopharynx, connected by the Eustachian tube, can be regarded as an interrelated dynamic system as well as a pathogenetic unit (Bluestone & Cantekin, 1981). The close correlation of the bacterial findings in the middle ear and in the nasopharynx in children with AOM is in full agreement with this opinion (Howie & Ploussard, 1971; Kamme et al., 1971; Nylén, 1975). Evidence has been presented that bacterial colonization in the nasopharynx is an important factor in SOM. It seems therefore important to eradicate the nasopharyngeal pathogens in the treatment of AOM and SOM in children. Penicillin has failed in this respect (Kamme et al., 1970). An antibiotic capable of eliminating respiratory pathogens not only in the middle ear but also in the nasopharynx is thus of interest.

Erythromycin has been used as an alternative to penicillin in the treatment of respiratory tract infections. In recent years, the prescription of erythromycin has considerably increased. Concomitantly, there has been an increased interest in the

pharmacokinetics of the drug (Axelsson & Brorson, 1974; Kalm et al., 1975; Paavolainen et al., 1977; Eliasson et al., 1978; Fraschini et al., 1978; Malmborg, 1978; Wollmer et al., 1982). However, the penetration of erythromycin into the middle ear and the adenoid has not been studied, neither has its effect on the nasopharyngeal flora.

A. Antibiotic penetration into the middle ear.

In an article on antibiotic penetration into middle ear, Silverstein et al. (1966) started with the following words: "To date, there is no proof that antibiotics reach the middle ear in effective concentrations during the course of active inflammation." This pioneer work in a field of research, previously untrodden, was followed by a series of studies on the penetration of various antibiotics to the middle ear in AOM and in SOM (Table I).

A common feature in penetration studies is the wide range of the results (Sabath, 1978), which is also evident in Table I. Although investigations of this kind have many similarities, the number of variables is still great: differences in age distribution, in dose and dose intervals, in interval between dose and sampling, in single or multiple dose procedures etc. In addition, methodological dissimilarities must be taken into account. Under these circumstances, the results of various studies should be compared with caution.

B. Antibiotic penetration into Waldeyer's ring.

There are surprisingly few studies performed on the penetration of antibiotics into the adenoid. *Spiramycin* penetrated to levels several times higher than the concomitant plasma levels

Table I. Literature survey of studies on the penetration of betalactam antibiotics and erythromycin to the middle ear in AOM and SOM.

Abbreviations: interv. = interval between the last dose and sampling; s = single dose; m = multiple doses; 50* = 50 mg/kg/day; ampi = ampicillin; amox = amoxicillin; azi = azidocillin; bac = bacampicillin; cefa = cefaclor; ery, ery-s and ery-e denote erythromycin, erythromycin ethylsuccinate and erythromycin estolate, respectively.

Author	Drug	Cases n:o	Adm.	Dose mg/kg	Interv. h	MEE mg/l	Plasma mg/l	MEE/ plasma
<u>AOM</u>								
Coffey Jr 1968	ampi	10	i.m.	35 s	1-1,5	9.5	15.4	0.62
Kamme et al 1969	pcV	25	oral	13 s	0.5-1.3	2.1	12.1	0.17
Kamme et al 1969	pcV	29	oral	26 s	0.5-1.3	6.3	15.5	0.40
Lahikainen 1970	pcV	65	i.m.	6 s	1	1.27	3.58	0.35
Bass et al 1971	ery-s	4	oral	50* m	2	0.84	1.30	0.65
Bass et al 1971	ery-e	4	oral	50* m	2	4.18	7.55	0.55
Lahikainen et al 1977	azi	15	oral	15 s	2	3.21	7.37	0.44
Lahikainen et al 1977	ampi	15	oral	10 s	2	2.17	4.30	0.49
Virtanen & Lahikainen 1979	bac	40	oral	13 s	1	2.4	7.7	0.31
<u>SOM</u>								
Lahikainen 1970	pcG	9	i.m.	6 s	2-4	<0.1	1.51	0
Lahikainen et al 1977	azi	18	oral	15 s	2	0.50	4.82	0.10
Lahikainen et al 1977	ampi	9	oral	10 s	2	0.23	3.62	0.06
Klimek et al 1977	amox	16	oral	40 s	1-2	6-2	15.3	0.41
Klimek et al 1977	ampi	13	oral	40 s	1-2	1.48	22.4	0.07
Krause et al 1982	amox	19	oral	15 s	2-3	5.6	5.8	0.97
Krause et al 1982	cefa	26	oral	15 s	0-0.5	3.8	12.8	0.30
Krause et al 1982	ery	15	oral	12.5 s	1-1.5	0	1.06	0
Edén et al 1983	cefa	41	oral	20 s	0.5	3.5	25	0.14
Edén et al 1983	cefa	23	oral	40 s	1.5-2	7.8	27.5	0.28

(Gaillard et al., 1971). *Clindamycin* showed a similar pattern of penetration with a mean tissue-to-plasma ratio of 250% (Panzer et al., 1972). *Benzylpenicillin*, by the intramuscular route of administration, gave concentrations in the order of 1/10th of the concomitant plasma levels, and after two hours no antibiotic could be detected (Sundén & Schiratzki, 1977). In a comprehensive study on the penetration of *cefactor*, the concentration in adenoid homogenates was in the order of 20-40% of the concomitant serum level (Edén et al., 1983).

Compared to the few studies on antibiotic penetration into adenoids, there is a plethora of investigations on antibiotic penetration into tonsils with a wide range in the obtained results (III. Table II). The tissue-to-plasma ratios refer to the quotient between the actual tissue concentration and the concomitant plasma level. When comparing the ratios, the range is conspicuously great, from 2-160% for penicillin and 13-275% for erythromycin. High values of the ratio are noteworthy and should arouse suspicion of possible artefacts.

Table II. The concentration of erythromycin in middle ear effusion and in plasma during a ten days course of treatment. Early input = 0-12 h; Late input = 24-36 h; Middle ear steady state = 48 h-10 days after initiated therapy. Output phase = 0-36 h after the last dose.

	Early input		Late input		Middle ear steady state		Output phase		
	2h	12h	24h	36h	48h	10d	12h	24h	36h
<i>Plasma</i>									
Mean mg/l	1.6	1.4	1.1	1.2	1.2	1.1	0.2	0.1	0.0
S.D.	0.69	1.1	0.5	0.6	0.9	0.8	0.2	0.1	
Range	0.5-3.0	0.5-4.2	0.6-2.0	0.2-1.8	0.4-3.2	0.0-4.2	0.0-0.4	0.0-0.3	
n	10	10	10	11	14	27	12	12	2
<i>Ear eff.</i>									
Mean mg/l	0.1	0.6	1.1	1.1	1.2	1.1	0.9	0.3	0.0
S.D.		0.5	0.7	0.5	0.6	0.9	0.7	0.3	
Range	0.0-0.4	0.3-1.5	0.3-2.2	0.2-2.3	0.4-2.7	0.4-4.2	0.0-2.5	0.0-0.8	
n	10	11	13	16	17	27	12	17	2
<i>Cultures</i>									
n	8	10	10	7	8	27	12	8	2

C. Secretory otitis media - a model to study antibiotic penetration.

The middle ear is in different ways very closely related to the respiratory system. *Anatomically*, it is a tissue-enclosed cavity, and the resemblance to the paranasal sinuses is striking. The *histology* of the normal middle ear mucosa is generally described as a modified respiratory mucosa of essentially the same appearance as the mucosa in other parts of the respiratory tract (Sadé, 1979). This is especially valid for the anterior parts of the middle ear, in the vicinity of the tubal orifice, where the mucosa is built up of a ciliated, cylindrical epithelium covered with a thin layer of mucus from goblet cells.

Respiratory mucosa, regardless of location, reacts in a uniform manner to an inflammatory stimulus by swelling, round cell infiltration in the submucosa and the development of numerous goblet cells and mucus glands, producing an abundance of secretion (Sadé, 1966; Sadé & Eliezer, 1970; Bak-Pedersen & Tos, 1971; Tos, 1980). This uniform mode of reaction occurs in the entire middle ear mucosa in SOM, in the sinus mucosa in chronic sinusitis and in the bronchial mucosa in chronic bronchitis. In addition, the same inflammatory pattern is observed in the mucosa during the resolution phase of AOM, acute sinusitis and bronchitis. Although the *histopathological* changes of the mucosa may vary quantitatively in different localities of the respiratory tract (Tos & Bak-Pedersen, 1973, 1976, 1977), it should be observed that the qualitative differences are indistinguishable for the pathologist (Sadé, 1979).

The *physiology* of respiration has been shown to apply to the middle ear (Flisberg, 1966) as well as to the paranasal sinuses (Aust & Drettner, 1974; Drettner & Aust, 1977). Bluestone & Cantekin (1981) pointed on the striking similarities between the trachea and the bronchi on one hand, and the Eustachian tube and the middle ear on the other. However, the pO_2 and pCO_2 in the open bronchial system are not always consistent with the conditions in cavities such as the paranasal sinuses and the middle ears.

According to *clinical* experience infections in different parts of the respiratory tract occur often simultaneously. The sino-bronchial syndrome is a well-known entity, the simultaneous infection of the middle ear and the paranasal sinuses another, the common source of infection being pathogens colonizing the nasopharynx.

There are obviously many reasons to regard SOM as a secretion-in-cavity model, suitable for the study of antibiotic penetration through inflamed respiratory mucosa. By analogy, the results should be valid for similarly inflamed mucosa in areas lined with a respiratory epithelium.

D. Microbiology of the middle ear effusion in children with secretory otitis media.

Although possibly described already 450 BC by Hippokrates (Pahor, 1978) SOM has in some respects remained an enigmatic disease. Thus, the etiological significance of the *respiratory pathogens* in the pathogenesis of SOM is still a controversial issue. On one end of the scale, bacteria in the MEE are merely looked upon as accidental findings without any importance, possibly residues from a previous AOM (Tos et al., 1979; Sipilä et al., 1981). On the other, bacteria are ascribed an etiological role in the development and maintenance of SOM (Liu et al., 1975; Giebink et al., 1979; Carenfelt, 1980).

When Politzer (1867) described a case of serous effusion behind an intact drum, this MEE was regarded as a *sterile* transudate according to the *hydrops ex vacuo theory*. On the other hand, the *inflammatory theory*, proposed by Brieger (1914), considered the MEE as an infectious exudate, produced by an inflammatory reaction of the mucosa (cited in Kokko, 1974). The validity of the two theories has been a matter of dispute for many years (Holmgren, 1940; Blegvad, 1941). During the last decade, the inflammatory theory has gained much support from

studies on the histological changes in the mucosa during the course of SOM and from the findings of inflammatory cells and immunoglobulins in the MEE (Sadé, 1966; Lewis et al., 1979; Sipilä & Karma, 1982).

The two theories may not be in conflict to each other, since several stimuli may evoke an inflammatory reaction of the mucosa. A negative intratympanic pressure or a change of the gas composition in the middle ear in anaerobic direction have been suggested to initiate an inflammatory reaction of the mucosa (Sadé, 1979; Tos et al., 1979). The results of animal experiments on chinchillas support this opinion. Thus, obstruction of the Eustachian tube first resulted in a serous MEE due to transudation from vessels in the subepithelial space, followed later in the course by an inflammatory reaction of the mucosa with a subsequent production of a mucoid MEE (Meyerhoff & Giebink, 1982).

When Senturia et al. (1958) succeeded in isolating bacteria in 41.5% of the MEEs in SOM, this finding was taken as a strong evidence of an *infectious* origin of the disease. The report has during the last decade, been confirmed by numerous studies, though there is a remarkably wide range in the bacterial recovery (e.g. Kokko, 1974; Liu et al., 1975; Healy & Teele, 1977; Riding et al., 1978; Giebink et al., 1979; Sipilä et al., 1981).

There seems to be a lack of consensus as to which bacteria should be classified as pathogens. *Staphylococcus epidermidis* and diphtheroids, normal inhabitants of the ear canal (Healy & Teele, 1977; Brook, 1981 a; Luotonen et al., 1982), have been regarded as contaminants, when found in the MEE. The pathogenicity of *Staphylococcus aureus* is still not clearly established, though there are reports supporting the classification of the organism as a non-pathogen in SOM (Kamme et al., 1971; Lewis et al., 1979).

The following bacteria, commonly classified as the main etiological agents in AOM, are classified as respiratory pathogens in SOM: *Streptococcus pneumoniae*, *Haemophilus influenzae*,

Branhamella catarrhalis and β -hemolytic streptococci. The demonstration of antibodies both in serum and in MEE in cases of OME has settled the question of the pathogenicity of *B. catarrhalis* (Leinonen et al., 1981). If this classification is applied to the studies mentioned above, the frequency of respiratory pathogens will range between 9-26%, compared to reported frequency of bacterial findings of 22-52%. In addition, non-viable bacteria have been found in smears of culture-negative MEEs. The significance of these intriguing findings is, as yet, a matter of speculation (Liu et al., 1975; Giebink et al., 1982; Meyerhoff & Giebink, 1982).

E. Microbiology of the nasopharynx in children with secretory otitis media.

The nasopharynx with the adenoid occupies a central part in the respiratory system and is in communication with both the oropharynx, the nasal cavities and the middle ears. Bacterial colonization takes primarily place in the secretions covering the adenoid (Lundberg & Lönnroth, 1979). The same pathogens as found in the secretions have also been described in the adenoid tissue itself (Ruokonen et al., 1979; Al-Sheikhli, 1980). However, there seems to be quantitative differences between pathogens colonizing in the tissue and pathogens in the secretions.

Nasopharyngeal cultures in healthy children yielded respiratory pathogens in 15-63% (Norstedt, 1967; Nylén, 1975; Ingvarsson et al., 1982), in otitis-prone children, but with no AOM at time of sampling, in 76% (Nylén, 1975) and in children with AOM in 55-98% (Howie & Ploussard, 1971; Kamme et al., 1971; Nylén, 1975; Qvarnberg, 1981; Virolainen et al., 1982). Although Ingvarsson et al. (cited in Lundgren & Rundcrantz, 1976) and Laurell et al. (1980) reported on nasopharyngeal pathogens in children with *SOM* in a frequency of about 80%, any comprehensive studies have not been published.

The phenomenon of bacterial *persistence* in the nasopharynx despite a seemingly adequate treatment with antibiotics has exclusively been studied in children with AOM. An investigation on the antibacterial activity on the nasopharyngeal pathogens in children with SOM has not yet been published, which is somewhat surprising since many children with SOM attend ENT-clinics, where facilities for sampling are easily available.

It is mainly the effect of betalactam antibiotics on respiratory pathogens in the nasopharynx of children with AOM, that has been studied. After treatment with ample doses of *penicillin*, *S. pneumoniae* survived in 15-67% and *H. influenzae* in 29-84% (Kamme et al., 1970; Herberts et al., 1971; Sprinkle & Veltri, 1971; Qvarnberg, 1981; Melén et al., 1982; Puhakka et al., 1982; Virolainen et al., 1982). Treatment with *azidocillin*, *ampicillin* and *cephalexin* decreased, transiently, the frequency of *S. pneumoniae* and group A streptococci, while *H. influenzae* was not influenced (Frölund Thomsen et al., 1976). While a ten days course of *amoxicillin* eradicated 94-100% of strains of *S. pneumoniae*, the survival rate of *H. influenzae* varied between 39-73% (Qvarnberg, 1981; Virolainen et al., 1982). After a similar course of *cefadroxil*, *S. pneumoniae* persisted in 36% and *H. influenzae* in 32% (Virolainen et al., 1982). When Qvarnberg (1981) treated twelve cases of AOM with *erythromycin*, reduction of nasopharyngeal pathogens was noted but the number of cases was too small to permit any definite conclusions to be drawn.

AIMS OF THE PRESENT INVESTIGATIONS

1. To study the penetration of erythromycin through middle ear mucosa in secretory otitis media (I).
2. To establish SOM as a model for the study of antibiotic penetration in inflamed respiratory mucosa (I).
3. To study the penetration of erythromycin in Waldeyer's ring (II, III).
4. To study the bacterial flora of respiratory pathogens in the middle ear and in the nasopharynx in children with persistent secretory otitis media (IV).
5. To study the effect of treatment with erythromycin on respiratory pathogens in the nasopharynx of children with persistent secretory otitis media (V).

THE PRESENT STUDIES

Penetration of erythromycin through respiratory mucosa (I).

This investigation was undertaken to study the penetration of erythromycin into the middle ear effusion of SOM.

Material and method

The study was based upon the participation of 108 patients with long-standing SOM, scheduled for myringotomy. The mean age was 13.1 years, range 1-68 years, 69% below the age of 10 years and 81% below 15 years of age. Erythromycin ethylsuccinate was given orally according to a dosage scheme (I. Table I). The patients were divided into nine groups, treated with erythromycin for various periods of time before operation, which was performed under general anaesthesia. MEE was collected, and a plasma sample was drawn simultaneously. The mean interval between the last dose and sampling was $2\frac{1}{2}$ hours except in the groups, where the elimination phase was studied.

Laboratory procedures

The erythromycin concentration in the MEE was determined against a plasma standard of the drug. The amount of MEE was too small to allow the use of a standard in this material in all the assays performed. A standard of pooled MEE was prepared and was used for comparison with the plasma standard. The regression lines from these two standards were not identical, but parallel, thereby making it possible to calculate the actual concentrations in the MEE samples from the plasma standard by using a correction factor of 1.4 (I. Table II).

Results

The results are presented as means, displayed in Fig. 1

and Table II. Erythromycin penetrated slowly into the MEE. In the early input phase, the concentration two hours after the last dose was below 0.1 mg/l, the lower limit of the assay method, and ten hours later, i.e. after the second dose, it was 0.6 mg/l. About one day after initiated treatment, i.e. after the fourth dose, a level of 1.1 mg/l was reached. This level was maintained at the determination of the concentration at 36 h, 48 h and on day ten. The elimination from the MEE was slow. The level twelve hours after the last dose following ten days of treatment was 0.9 mg/l, and after a further twelve hours 0.3 mg/l. No erythromycin was detected 36 hours after the last dose.

The concentration in *plasma* was 1.1-1.6 mg/l about two hours after every dose (Table II). The elimination was considerably more rapid from plasma than from MEE; thus, the plasma concentration 12 hours after the last dose was 0.2 mg/l. In Table III, the relation between the concentration of erythromycin in MEE and the concomitant plasma level is shown.

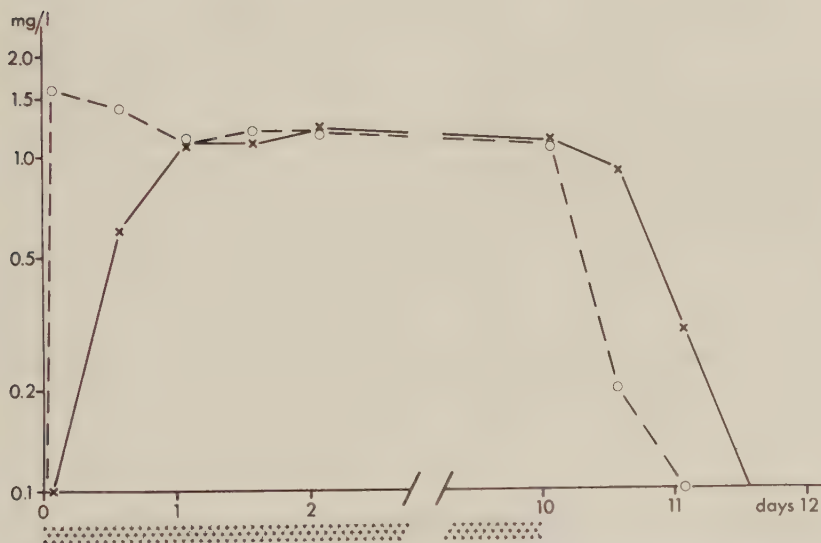


Fig. 1. Erythromycin concentration in the middle ear effusion and in plasma. Continuous line: concentration in middle ear effusion. Broken line: concentration in plasma. All values shown are means of 10 or more determinations except on day 11½, where there are only two cases. Shaded area denotes erythromycin therapy.

Table III. *The ratio (%) between the concentration of erythromycin in the middle ear effusion and the concomitant plasma sample during ten days of treatment and 1½ day after its completion (elimination phase).*

Treatment period							Elimination phase		
time	2h	12h	24h	36h	48h	day 10	12h	24h	36h
MEE/plasma	6	40	100	90	100	100	450	300	0

Table IV. *The concentration of erythromycin in blood-free adenoid tissue homogenates and in plasma during 4 days of treatment. Input = 3 hours; Output = 12 h after the last dose.*

	Input					Output
	3h	12h	24h	36h	48h	12h
Plasma						
Mean, mg/l	1.2	0.8	0.8	0.8	0.9	0.2
SD	0.7	0.9	0.4	0.6	0.6	
Range	0.4-2.4	0.1-3.6	0.1-1.6	0.2-2.3	0.1-2.2	0.0-0.6
Adenoid tissue						
Mean, mg/l	1.3	0.9	1.1	1.3	1.1	0.1
SD	1.1	0.8	0.9	0.9	0.7	
Range	0.2-3.7	0.2-2.4	0.2-3.4	0.4-3.5	0.2-2.7	0.0-0.4
n	12	12	11	15	12	10

Penetration of erythromycin in Waldeyer's ring - adenoid tissue (II).

The aim of the present investigation was to study the penetration of erythromycin into adenoid tissue.

Material and method

The study was based upon the participation of 72 children, where adenoidectomy was indicated due to nasal obstruction by an enlarged adenoid. Erythromycin ethylsuccinate was given orally according to a dosage scheme (II. Table II) for different intervals of time before scheduled operation. The children were divided into different groups, operated upon after the first, the second, the third, the fourth and the fifth dose. The interval between the last dose and sampling ranged between 1.9-3.1 hours (mean 2.5 ± 0.7) except when the elimination was studied. Tissue samples, plasma and whole blood samples were collected in association with adenoidectomy.

Laboratory procedures

Series of standards were made on adenoid tissue homogenates, on plasma and on whole blood. Since the regression lines from the whole blood standard and the tissue standard were identical in repeated experiments, the concentration of erythromycin in samples of adenoid tissue homogenate could be calculated from the whole blood standard.

The following formula was used to calculate the concentration of erythromycin in blood-free tissue homogenates:

$$C_t = \frac{C_s - C_b \cdot k}{1 - k}$$

C_t , C_s and C_b denote the concentration of erythromycin in blood-free tissue, in sample and in whole blood, respectively. The coefficient of blood contamination (k) is given by the ratio between haemoglobin in tissue homogenate and in whole blood. The derivation of the formula is described in Appendix of paper IV.

Results

The results, presented in Fig. 2 and Table IV, are all means and represent blood-free tissue homogenate concentrations. The penetration of erythromycin was rapid; the tissue concentration three hours after the first dose was 1.3 mg/l, which was almost the same as the concomitant plasma level. During the continued course of therapy, the tissue concentration, two to three hours after the second to fifth doses, varied between 0.9-1.3 mg/l and the concomitant plasma concentrations between 0.8-0.9 mg/l. The elimination was rapid. The concentration in tissue and in plasma was below the lower limit of the assay method twelve hours after the last dose. The close correlation of the concentration of erythromycin in tissue and in concomitant plasma samples is shown in Fig. 3. The blood contamination in adenoid tissue was in the order of 6%.

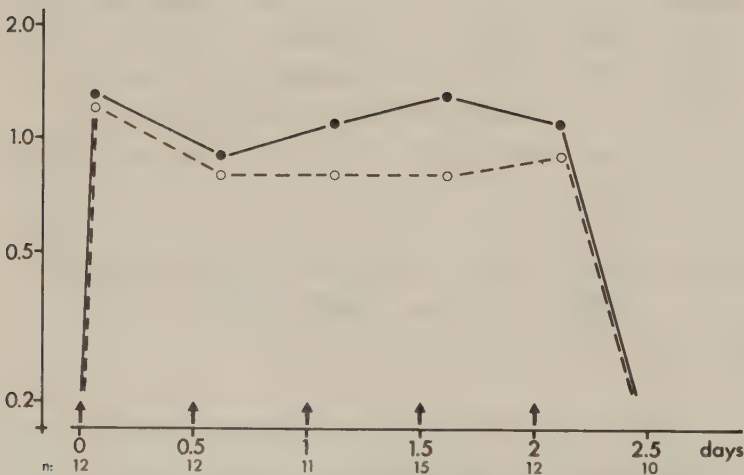


Fig. 2. Erythromycin penetration into adenoid tissue, given as mean values for blood-free tissue homogenates. The continuous line shows the tissue levels, the broken line shows the plasma values. Arrows indicate doses; n = number of cases in each group.

Penetration of erythromycin in Waldeyer's ring - tonsil tissue (III).

The investigation was undertaken to study the penetration of erythromycin into tonsil tissue.

Material and method

The study included 26 patients, scheduled for tonsillectomy under the diagnosis of chronic tonsillitis. In the first part of the study, erythromycin ethylsuccinate was administered orally to twenty patients twice a day, in a mean dose of 39 mg/kg (range 24-74 mg/kg), for different periods of time prior to tonsillectomy. Tissue samples, plasma and whole blood samples were collected at operation, in six cases after one dose, in four cases after two doses, in nine cases after three doses and in one case after five doses (Table V). The mean interval between the last dose and sampling was 3.4 hours (range 2.3-5.0 hours). In the second part of the study, com-

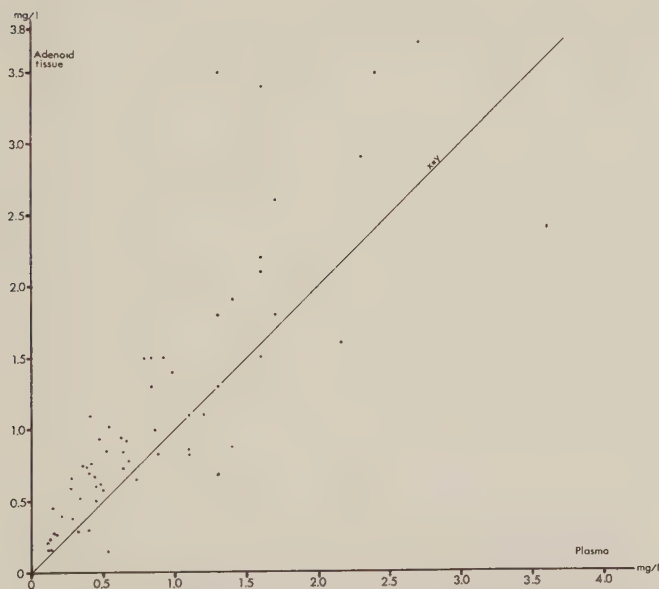


Fig. 3. Diagram showing the correlation between the concentration of erythromycin in blood-free adenoid tissue homogenate and in concomitantly drawn plasma samples. Every individual case is marked by a dot; $n = 62$.

prising six patients, erythromycin ethylsuccinate was given via a gastric tube to prevent surface contamination, three to five hours before operation and sampling. In addition, the absorption was monitored by frequent plasma analyses. The same laboratory procedure was employed as in study II.

Results

The results, presented in Fig. 4 and Fig. 5 and in Table V, are all means and refer to concentrations in blood-free tissue homogenates. In the first part of the investigation (Fig. 4 and Table V) the mean concentration (M) of erythromycin in tonsil tissue varied between 1.3-1.4 mg/l and in the concomitant plasma concentration between 0.7-0.9 mg/l. Since the tissue-to-plasma ratio was about 200% (Table V) an artefact was suspected in the group of patients, where erythromycin was administered orally. In the second part of the study, six patients received erythromycin ethylsuccinate via a gastric tube. The results showed now similar concentrations in tissue and in the concomitant plasma samples with a tissue-to-plasma ratio of 100% (Fig. 5 and Table V). The tissue values tallied very well with the results in adenoid tissue (II). The blood contamination was determined to 10%.

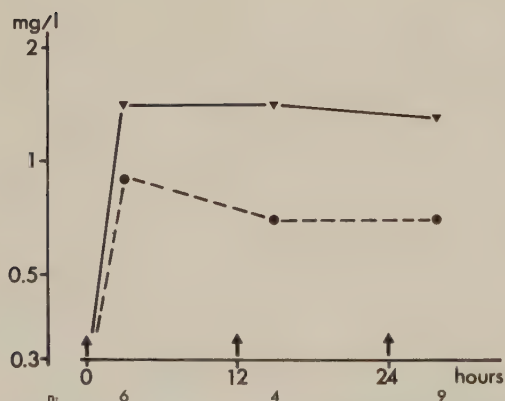


Fig. 4. Concentration of orally given erythromycin into tonsil tissue and plasma. The continuous line denotes erythromycin concentration in blood-free tissue homogenates; the broken line shows the erythromycin level in plasma. Arrows indicate the 1st, 2nd and 3rd dose of 1000 mg erythromycin. The values are means and n shows the number of cases in each group.

Table V. The concentration of erythromycin in blood-free tonsil tissue homogenates and in plasma in relation to the number of given doses. Twenty patients received erythromycin orally and six patients via a gastric tube. M = mean in respective group.

Patient no.	Doses no.	Route	Plasma mg/l	Tonsil A mg/l	Tonsil B mg/l	Tonsil/plasma ratio %	Time after given dose
1	1	Oral	0.2	0.6	0.6		4.0
2	1	Oral	0.3	0.2	0.2		3.8
3	1	Oral	0.8	2.0	2.0		2.5
4	1	Oral	0.9	1.5	1.4		3.0
5	1	Oral	1.2	1.6	1.6		3.5
6	1	Oral	1.7 M:0.9	2.7	2.7 M:1.4	155	2.7 M:3.25
7	2	Oral	0.2	0.6	0.6		3.3
8	2	Oral	0.5	0.9	0.9		2.8
9	2	Oral	1.0	2.8	2.7		2.8
10	2	Oral	1.1 M:0.7	1.3	1.3 M:1.4	200	2.3 M:2.80
11	3	Oral	0.2	0.6	0.5		3.8
12	3	Oral	0.3	1.0	1.0		2.5
13	3	Oral	0.5	0.6	0.6		5.0
14	3	Oral	0.5	1.0	0.8		3.9
15	3	Oral	0.6	1.3	1.3		3.4
16	3	Oral	0.7	1.3	1.3		3.5
17	3	Oral	1.0	1.6	1.6		3.3
18	3	Oral	1.3	2.0	2.0		3.1
19	3	Oral	1.5 M:0.7	2.4	2.2 M:1.3	186	3.5 M:3.56
20	5	Oral	0.4	0.6	0.6		4.7
1	1	Via tube	0.2	0.2	0.2		4.3
2	1	Via tube	0.3	0.4	0.4		3.9
3	1	Via tube	0.3	0.4	0.4		4.9
4	1	Via tube	0.6	0.6	0.6		4.0
5	1	Via tube	1.2	1.6	1.6		3.8
6	1	Via tube	1.3 M:0.7	1.1	0.9 M:0.7	100	3.3 M:4.03

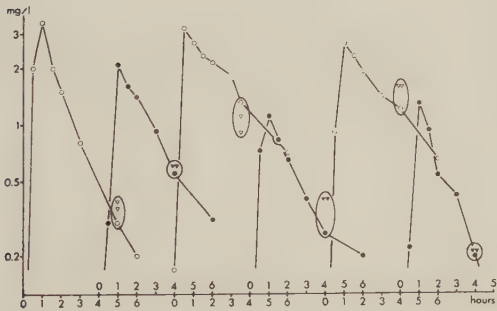


Fig. 5. Erythromycin penetration into tonsil tissue. The composite graph shows the plasma levels in six patients during the first six hours after a single dose of 1000 mg erythromycin administered via a gastric tube. The plasma levels and the corresponding blood-free tonsil tissue levels are shown inside each oval. Circles = plasma levels. Triangles = tonsil tissue levels. The symbols of alternate patients are filled for reasons of clarity.

Bacteriology of secretory otitis media (IV).

The present investigation was undertaken to study the occurrence of respiratory pathogens in the nasopharynx and in the middle ear of children with persistent SOM.

Material and method

Totally 92 children with SOM, persistent for three months or more, participated in the study (mean age 6.25 years, range 1-16 years). *Nasopharyngeal cultures* were taken from 76 children. Sampling was performed under general anaesthesia, allowing the swab to stay on the adenoid for at least ten seconds. In a partly overlapping series, *cultures of MEEs* were recovered from 61 children. Myringotomy and sampling were performed under the control of microscope with the patient under general anaesthesia.

Results

The results are displayed in Table VI. Nasopharyngeal cultures in persistent SOM yielded respiratory pathogens in 60 cases (79%). *S. pneumoniae* was recovered in 40 cultures (53%), *H. influenzae* in 38 cultures (50%) and *B. catarrhalis* in 25 cultures (33%). Streptococci group A were obtained in one case. A single pathogen was isolated in 24 cases (32%), two pathogens in 28 cases (37%) and three pathogens in 8 cases (11%).

Cultures of MEEs yielded respiratory pathogens in 11 cases (18%). *H. influenzae* was isolated in 7 cases, *S. pneumoniae* in 1 case and *B. catarrhalis* in 2 cases, and in 1 case all three of these pathogens were found.

Cultures from *both* the nasopharynx and the middle ear were made in 44 cases. Pathogens were obtained in both localities in 8 cases, in none of the localities in 7 cases, and in the nasopharynx solely in 29 cases. Respiratory pathogens, present in the middle ear, could always be isolated in the nasopharynx, either in pure or mixed cultures (IV. Table III).

Table VI. Bacterial findings in the nasopharynx of 76 children and in the middle ear effusions of 61 ears of 61 children with secretory otitis media. Number of cases.

	Naso-pharynx	Middle ear
<i>Haemophilus influenzae</i>	12 (1)	7
<i>Streptococcus pneumoniae</i>	6	1
<i>Branhamella catarrhalis</i>	6 (4)	2
<i>Haemophilus influenzae</i> }	16	
<i>Streptococcus pneumoniae</i> }		
<i>Haemophilus influenzae</i> }	2	
<i>Branhamella catarrhalis</i> }		
<i>Streptococcus pneumoniae</i> }	10	
<i>Branhamella catarrhalis</i> }		
<i>Haemophilus influenzae</i> }	7	1
<i>Streptococcus pneumoniae</i> }		
<i>Branhamella catarrhalis</i> }		
<i>Haemophilus influenzae</i> }	1	
<i>Streptococcus pneumoniae</i> }		
<i>Haemolytic streptococci A</i> }		
Respir. pathogens total	60	11
No growth of respir.path.	16 Σ 76	50 Σ 61
Isolates of non-pathogens:		
<i>Staphylococcus epidermidis</i>	13	9
<i>Staphylococcus aureus</i>	13 (11)	1 (1)
<i>Streptococcus viridans</i>	14	1
<i>Neisseria</i> sp.	5	1
<i>Haemophilus parainfluenzae</i>	1	

() = beta-lactamase producing.

Table VII. Comparisons of the nasopharyngeal findings in children treated with erythromycin for ten days (n = 38) and a control group of children (n = 37) not receiving antibiotics.

Pathogens	Control group	Treated group	χ^2 value	Level of significance
No pathogen	5	20	11.384	p<0.001
One pathogen	15	18	0.150	p>0.5
Two or more pathogens	17	0	20.296	p<0.001
<i>S. pneumoniae</i>	13	1	11.202	p<0.001
<i>B. catarrhalis</i>	12	0	12.595	p<0.001
<i>H. influenzae</i>	25	17	3.181	p>0.05
β -hemolytic streptococci	2	0		

The effect of erythromycin on the nasopharyngeal pathogens in children with secretory otitis media (V).

The aim of the present investigation was to study the effect of erythromycin on the respiratory pathogens in the nasopharynx of children with persistent SOM.

Material and method

Nasopharyngeal cultures were obtained from 75 children, not older than eleven years, with SOM for at least three months. The children were randomly divided into two groups before myringotomy. One group (n = 38) was treated with erythromycin ethylsuccinate orally, 20-30 mg/kg twice a day during ten days prior to scheduled operation, while a control group (n = 37) received no antibiotics before surgery. Sampling was performed in the same manner as in study IV.

Results

The results are shown in Table VII. Respiratory pathogens were found in 32/37 children (86%) in the *control* group. The following isolates were recovered: 25 isolates of *H. influenzae*, 12 isolates of *B. catarrhalis*, 13 isolates of *S. pneumoniae* and 2 isolates of β -hemolytic streptococci (group A and G), totally 52 isolates.

The nasopharyngeal cultures in the group, *treated with erythromycin*, differed from the control group in the following respects: the *total isolation rate* of respiratory pathogens was 18/38 samples, i.e. significantly lower (47% vs 86%), as was the finding of two or more pathogens in the same sample, 0/17 (0% vs 46%). The number of cultures yielding growth of *S. pneumoniae* and *B. catarrhalis* was lower in the treated group as compared to the control group, 1/13 and 0/12 (3% vs 35% and 0% vs 32%, respectively).

The occurrence of *H. influenzae* was not significantly lower in the group, treated with erythromycin: 17/38 compared to 25/37 in the control group. No β -hemolytic streptococci were isolated in the erythromycin-treated group.

GENERAL DISCUSSION

A. Penetration of erythromycin into the middle ear and Waldeyer's ring.

Erythromycin is a common alternative to penicillin in the treatment of respiratory tract infections in children. However, still 30 years after its introduction in clinical practice, there is a lack of knowledge about the penetration characteristics of erythromycin in important parts of the respiratory tract. Therefore, the penetration into the middle ear and to Waldeyer's ring has been studied in the present investigations (I, II and III).

There has been a tendency during the last ten years to reduce the number of daily *doses* from three to two (Rundcrantz & Sundför, 1974). In study I, erythromycin was administered three times a day (morning, noon and evening), while in studies II and III a two-dose procedure was applied. However, the total dose per day and per kg bodyweight in the studied groups was the same (I. Table I; II. Table II). In addition, despite the difference in the daily dose between children (42-60 mg/kg bodyweight) and adults (18-62 mg/kg bodyweight), the concentration of erythromycin in the MEE during the course of medication was the same in the different age groups (I. Table VI).

Blood contamination is a source of error when determining the tissue concentration of antibiotics, especially when the tissue level differs markedly from that in blood. In contrast to most studies, the present results are corrected for blood contamination, being 6% in adenoid tissue and 10% in tonsil tissue, which is in agreement with other studies (Ekedahl et al., 1978).

Another possible source of error, never described before, is *surface contamination* of the tonsils, which occurs when mixtures of antibiotics have been swallowed. The magnitude of

this pitfall was elucidated in study III, where the tissue-to-plasma ratio was about 100% higher, when erythromycin was swallowed, than the corresponding ratio, when erythromycin was administered via a gastric tube (Fig. IV, Fig. V). This surface contamination of antibiotics may not, however, be waived, since it probably exerts an antibacterial activity on pathogens on the surface of the tonsils. The adenoid is protected by the soft palate and is thus not exposed to this kind of contamination.

Agar well diffusion technique is the standard method in determining antibiotic concentrations in body fluids, including the sometimes very viscous respiratory secretions (Kalm et al., 1975). The method requires antibiotic standards prepared in solutions with the same properties as the investigated samples to give reliable results (Kaplan et al., 1973; Ögren & Cars, 1982). Ideally, MEE samples should be assayed by means of standards in like material, i.e. MEE of untreated patients with SOM. This was, however, not possible, since the amount of such MEE did not suffice for the great number of determinations required for the study. The problem was solved by using plasma standards, whose regression lines were, not identical with, but parallel to the regression lines for standards prepared in MEE. Due to the relationship between the standards, the concentration of erythromycin in the MEE samples could be calculated from the plasma standards by a correction factor of 1.4 (I. Table II).

Unavoidably, there are some errors related to the method in this kind of assay. The coefficient of variation (SD in % of means) in plasma was about 10% (I. Table III). The corresponding value in MEE ranged between 13-26% (I. Table IV), which during the course of calculation was multiplied by the correction factor of 1.4. The higher coefficient of variation in MEE could partly be explained by difficulties to fill the wells right up to the brim with an often very viscous secretion. However, in practice, the accuracy of the method was considered adequate for the purpose of the study. This was also confirmed by a good agreement of results from samples obtained from both ears of the same patient.

The agar well diffusion technique was also used to determine the concentration of erythromycin in adenoid and tonsil tissue homogenates (II, III). The regression lines of the whole blood standard and the tissue standard were identical. The concentration of erythromycin in samples from adenoid and tonsil tissue was therefore calculated from the whole blood standard, and any correction factor was not needed.

A common feature in penetration studies is the wide *range* of the results (Sabath, 1978). There are numerous variables involved in this kind of investigations, e.g. individual differences in absorption. However, the range of the results in the present studies (I, II, III) was not larger than what could be expected, as judged from other studies on the penetration of antibiotics (e.g. Lahikainen et al., 1977; Krause et al., 1982).

Since erythromycin penetrates tissues and secretions by means of i.a. passive diffusion (Dette, 1979), the concentration gradient will be of great importance for the distribution. When erythromycin penetrated into the MEE, the concentration of the drug was higher in the blood than in the MEE (Table II). Eneroth & Lundberg (1976) found a similar pattern of concentration when studying antibiotic penetration from blood into sinus secretion. In the elimination phase, the level of erythromycin was higher in the MEE than in blood (Table II). Except by rediffusion, there is a possibility, that erythromycin might be metabolized in the MEE or eliminated together with MEE via mucociliary clearance. These mechanisms of elimination have, however, not yet been clarified.

The *penetration* of erythromycin into MEE was remarkably slow. The highest level in the determinations of the concentration level (Fig. 1) was not reached until after about 24 hours or four doses. This is in contrast to other antibiotics, which reach the highest concentration in the MEE already within a couple of hours after a single dose (Sundberg et al., 1978; Klimek et al., 1980; Krause et al., 1982; Edén et al., 1983; Sundberg et al., 1983).

The results of a single dose procedure, when studying antibiotic penetration into the MEE, can thus be misleading for antibiotics with a slow penetration rate. For example, Krause et al. (1982) were surprised not to find erythromycin in the MEE 30 to 240 minutes after the administration of a single dose. This was, however, not remarkable with the knowledge of the slow penetration rate, clearly demonstrated in study I, and emphasizes the importance of using a multiple dose procedure to elucidate the whole course of penetration.

During the continued medication, the concentration of erythromycin in the MEE was found to be 1.0 mg/l on different occasions (Fig. 1). The elimination, being a mirror of the input phase, was not completed until after 24 hours after the last dose. Thus, from about 24 hours after initiated treatment to about 12 hours after its completion, the level of erythromycin in the MEE was sustained at a rather even level of about 1 mg/l and did not follow the fluctuations in plasma.

The concentration of penicillin in the MEE of AOM decreased considerably after about two days of treatment despite high plasma levels (Lundgren & Rundcrantz, 1976; Ingvarsson et al., 1980). In established SOM, the concentration of penicillin was 1/10 of that in the early phase of AOM (Lahikainen, 1970). Thus, in this phase of active inflammation, when hyperemia and exudation are prominent features in the mucosa, penicillin passed very readily into the MEE (Ingvarsson et al., 1980). However, later in the course of AOM, when the acute inflammation subsided, the concentration of penicillin was only about 20% of the corresponding plasma level, thus being at about the same level as found in SOM (Lahikainen, 1970). In contrast to this, the concentration of erythromycin in the MEE of SOM during the course of medication was similar to the concomitant plasma levels (Fig. 1; Table II), i.e. a MEE-to-plasma ratio of 100% (Table III). Thus, in this respect, there was noted a very interesting difference in the penetration pattern of penicillin and that of erythromycin. It is a matter of speculation, if it is the histopathological changes in the middle ear mucosa and changes of the viscosity of the MEE during the

course of inflammation, which make the penetration into MEE more difficult for penicillin than for erythromycin.

Studies on antibiotic penetration through inflamed respiratory mucosa in other parts of the respiratory tract have been performed, mainly in the paranasal sinuses (Lundberg et al., 1968; Axelsson & Brorson, 1974; Eneroth et al., 1975; Kalm et al., 1975; Paavolainen et al., 1977; Sundberg et al., 1983) but also in patients with chronic bronchitis (Fraschini et al., 1978). The results of these studies are in agreement with the concentrations achieved by the *SOM model* and lend support to the concept that antibiotic penetration through inflamed respiratory mucosa is largely independent of its location.

The SOM model offers several advantages in the study of antibiotic penetration in the respiratory tract. Myringotomy with insertion of a grommet is the standard procedure in patients with SOM, who do not respond to conservative treatment. Since the MEE does not recur as long as the inserted tube is in function, we are confined to one sample of MEE per patient. However, SOM is a common condition, that frequently requires surgical intervention. At grommet insertion, the MEE is always aspirated, and samples are thereby achieved without extra procedures. In addition, contamination of the MEE with blood seldom occurs and is easily detected in the otomicroscope.

The *penetration into adenoid tissue* was rapid, in contrast to the slow penetration into the MEE, indicating absence of barriers for antibiotic penetration. The parallelity between the tissue concentration in blood-free homogenates and the concomitant plasma concentration is typical, when the penetration path is short, e.g. from blood to interstitial fluid (Barza, 1981). It should, however, be observed that the concentrations in tissue homogenates do not demonstrate the actual tissue distribution of the drug *in vivo*.

The results on the *penetration of erythromycin into tonsil tissue* (III) are completely in accordance with the results obtained on adenoid penetration (II). However, the number of

cases do not permit more far-reaching conclusions.

In sum, a satisfactory distribution of erythromycin into tissues and secretions has been demonstrated (I, II, III) - evident by the obtained tissue-to-plasma ratios and the MEE-to-plasma ratios (Table III and V). These findings are consonant with other studies, e.g. on the penetration of erythromycin to bronchial secretion and pulmonary tissue (Fraschini et al., 1978; Wollmer et al., 1982). The obtained concentrations of erythromycin in the MEE and in the adenoid and tonsil tissues surpassed the MICs of most respiratory pathogens with the exceptions of many strains of *H. influenzae*.

B. Microbiology of the middle ear and the nasopharynx in children with secretory otitis media.

The colonization of respiratory pathogens in the nasopharynx of children seems to constitute a potential source of infection to various parts of the respiratory system (Howie & Ploussard, 1971; Brorson & Malmvall, 1981). The close correlation between pathogens in the nasopharynx and in the middle ear in children with AOM has e.g. been convincingly demonstrated (Kamme et al., 1970). In otitis-prone but healthy children nasopharyngeal pathogens were isolated in 76% compared to 15% in children with occasional episodes of AOM (Nylén, 1975).

Any comprehensive study on the colonization of respiratory pathogens in the nasopharynx of children with SOM has, as yet, not been published. In the present investigations (IV, V), nasopharyngeal cultures yielded respiratory pathogens in 79% (IV) and 86% (V), respectively. It should be emphasized that the inclusion criteria were well-defined and an explicit diagnosis of SOM persistent for at least three months was required. Sampling was performed under ideally controlled conditions with the patient under general anaesthesia. The recovery rate was almost the same, as reported by Ingvarsson et

al. (84%) in an unpublished study (cited in Lundgren & Rundcrantz (1976), and by Laurell et al. (1980) (84%). In the latter material the duration of SOM was from one to six months.

Cultures of MEEs in children with persistent SOM yielded pathogens in 18%, which is consonant with other studies (Kokko, 1974; Healy & Teele, 1977; Giebink et al., 1979), when the same definition on respiratory pathogens is used as in the present studies (see page 14). When pathogens were present in the middle ear they could always be recovered also from the nasopharynx, either in pure culture or in mixed culture (V. Table III). Aspiration or insufflation of nasopharyngeal secretions, harbouring pathogens, are probably the mechanism by which these pathogens arrive at the middle ear (Smyth, 1978; Lim & DeMaria, 1982).

The number of sterile MEEs in SOM was 82% (IV), which is a considerably higher frequency than usually is found in AOM. It has been claimed, that immunoglobulins, lysozymes, phagocytes etc. probably can turn bacterial MEEs sterile and perhaps also prevent a recolonization of the MEE by pathogens. This is also consistent with the findings of a higher frequency of sterile MEEs in older children, where the immune defence is more mature (Liu et al., 1975).

The effect of betalactam antibiotics on nasopharyngeal pathogens in children with AOM is not satisfactory with a remarkably high frequency of persistent strains of *S. pneumoniae* and *H. influenzae* after a seemingly adequate treatment (Sprinkle & Veltri, 1971; Qvarnberg, 1981; Virolainen et al., 1982). The mechanism behind this bacterial persistence is not known. It has been claimed that local factors may decrease the activity of a drug: enzymatic inactivation of antibiotics at the site of infection could be one mechanism (Acar & Sabath, 1978). This possibility is consistent with reports on an increased number of betalactamase producing aerobes and anaerobes, e.g. isolated in Waldayer's ring in a frequency of up to 80% (Brook, 1981 b; Brook et al., 1981; Tunér & Nord, 1983) while the frequency of strains of *H. influenzae*, producing beta-

lactamase, has been fairly unchanged during the last years, the increase of betalactamase producing strains of *B. catarrhalis* could be described as explosive (Kamme, 1980; Michaels, 1981). Brook (1981 b) has described the picture of penicillin-sensitive bacteria, protected behind a "shield" of enzymes, "disarming" betalactam antibiotics. Erythromycin as a macrolide should not be inactivated by betalactamase in contrast to betalactam antibiotics.

The effect of ten days treatment with erythromycin was a significant decrease of *S. pneumoniae* and *B. catarrhalis* compared to a control group, while there was no significant influence on *H. influenzae* in the treatment group. These results are in full accordance with our previous penetration studies (I, II), where the concentration of erythromycin in blood-free adenoid tissue homogenates and MEE surpassed the MICs of all respiratory pathogens but many strains of *H. influenzae*.

In sum, children with persistent SOM harboured respiratory pathogens in the nasopharynx in about 80% and in the middle ear effusion in 18%. A ten days course of erythromycin in children with persistent SOM, almost eliminated *S. pneumoniae* and *B. catarrhalis* from the nasopharynx. The frequency of *H. influenzae* was not significantly influenced. The results are consonant with the results of our studies on the penetration of erythromycin in the MEE and in the adenoid tissue (I, II).

SUMMARY

The penetration of erythromycin through inflamed middle ear mucosa was studied in 108 cases of long-standing SOM. The concentration in the MEE increased slowly. About 24 hours after initiated therapy, i.e. after the fourth dose, the concentration reached a level of about 1.0 mg/l which was maintained during the continued course of medication. The elimination was considerably slower from the MEE than from plasma. The concentration of erythromycin achieved in the MEE surpassed with a wide margin the MICs of *S. pneumoniae*, *B. catarrhalis* and group A streptococci but not the MIC of many strains of *H. influenzae* (I).

SOM has been used as a secretion-in-cavity model to study antibiotic penetration through inflamed middle ear mucosa. The results of such a penetration study may be assumed to apply to inflamed respiratory mucosa independent of its location in the respiratory system (I).

The penetration of erythromycin into blood-free adenoid (II) and tonsil (III) tissue homogenates was investigated in 72 children with adenoid enlargement and in 26 patients with chronic tonsillitis. The penetration was rapid and similar in adenoid and tonsil tissues. The concentrations in tissue were almost equal to the concomitant plasma levels. The concentration of erythromycin in adenoid and tonsil tissues surpassed the MICs of *S. pneumoniae*, *B. catarrhalis* and group A streptococci but not the MIC of many strains of *H. influenzae*.

Nasopharyngeal cultures were obtained under general anaesthesia from 76 children with persistent SOM. Respiratory pathogens were recovered from 79% of the cases. *S. pneumoniae* was isolated in 53%, *H. influenzae* in 50% and *B. catarrhalis* in 33%. In a partly overlapping series, cultures from MEEs of 61 children with persistent SOM yielded respiratory pathogens

in 18%. Cases with pathogens in the middle ear had always the corresponding pathogen in the nasopharynx (IV).

Nasopharyngeal cultures of 38 children with persistent SOM, treated with erythromycin for ten days prior to sampling were compared to nasopharyngeal cultures from a control group of 37 non-treated children. The total isolation rate of respiratory pathogens was significantly lower in the group treated with erythromycin (47% vs 86%), as was the number of cultures yielding *S. pneumoniae* (3% vs 35%) and *B. catarrhalis* (0% vs 32%). The frequency of *H. influenzae* was not significantly influenced by treatment with erythromycin (V).

ZUSAMMENFASSUNG

Die Penetration von Erythromycin in die entzündete Mittelohrschleimhaut wurde an 108 Patienten mit einer persistierenden Otosalpingitis untersucht. Die Konzentration im Mittelohrexsudat stieg langsam an, etwa 24 Stunden nach Beginn der Behandlung, bzw. nach der vierten Dosis, erreichte die Konzentrationskurve ein Plateau von etwa 1,0 mg/l. Die "steady-state" Konzentration blieb auch während der weiteren Behandlung auf einem konstanten Niveau.

Die Ausscheidung verlief wesentlich langsamer aus dem Mittelohrexsudat, als aus dem Plasma. Die Konzentrationen von Erythromycin in den Mittelohrexsudaten überschritten mit sicherem Marginal die MIC von *S. pneumoniae*, *B. catarrhalis* und Streptokokken, erreichte jedoch nicht die MIC einer grösseren Anzahl Stämme von *H. influenzae*.

Die Otosalpingitis wurde als Modell für die Sekretion in einen präformierten Hohlraum gewählt, um die Antibiotikapenetration durch die entzündete Mittelohrschleimhaut zu studieren. Die Resultate dieser Penetrationsstudie sind allgemeingültig für die entzündete respiratorische Schleimhaut, unabhängig von ihrer Lokalisation im Respirationstrakt.

Die Penetration von Erythromycin in blutfreie Gewebekomponenten aus Rachen- und Gaumenmandeln wurden an 72 Kinder mit einer Rachenmandelhyperplasie und an 26 Patienten mit einer chronischen Tonsillitis untersucht. Die Penetration verlief rasch und war gleichartig für Rachen- und Gaumenmandeln. Die Gewebekonzentrationen waren so gut wie identisch mit den Plasmakonzentrationen. Die Ausscheidung aus dem Gewebe verlief genau so schnell wie die Penetration. Die Konzentration von Erythromycin in dem Gewebe der Rachen- und Gaumenmandeln überschritt die MIC für *S. pneumoniae*, *B. catarrhalis* und Streptokokken. Die MIC einer grossen Anzahl Stämme von *H. influenzae* wurde jedoch

nicht erreicht.

Nasopharyngeale Kulturen wurden von 76 Kindern mit einer persistierenden Otosalpingitis unter Narkose entnommen. In 79% der Fälle fand man pathogene Bakterienstämme. *S. pneumoniae* wurde in 53% isoliert, *H. influenzae* in 50% und *B. catarrhalis* in 33%. In teilweise sich überschneidenden Serien fand man in Kulturen des Mittelohrexsudates von 61 Kindern mit persistierender Otosalpingitis pathogene Bakterienstämme in 18%. Bei Kindern die pathogene Bakterienstämme im Mittelohr hatten, fand man immer die korrespondierenden Stämme im Nasopharynx.

Nasopharyngeale Bakterienkulturen von 38 Kindern, die zehn Tage lang vor der Probeentnahme mit Erythromycin behandelt worden waren, wurden mit Nasopharynxkulturen einer nicht behandelten Kontrollgruppe von 37 Kindern verglichen. Die totale Anzahl isolierter pathogener Bakterienstämme war signifikant niedriger in der mit Erythromycin behandelte Gruppe (47% vs 86%); ebenso die Anzahl Bakterienkulturen die *S. pneumoniae* (3% vs 35%) und *B. catarrhalis* (0% vs 32%) aufwiesen. Das Vorkommen von *H. influenzae* wurde durch die Behandlung mit Erythromycin nicht signifikant beeinflusst.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Professor Carl-Magnus Eneroth, Head of the Department of Oto-Rhino-Laryngology, University Hospital, Lund, for stimulating encouragement, generous support and constructive criticism.

Docent Stefan Ernstson, my teacher, for inspiring guidance, fruitful discussions and critical and constructive suggestions.

Dr Tony Edén for expert guidance in the methodological parts and constructive criticism.

Dr Karin Prellner for valuable advice and constructive criticism of the manuscript.

Dr Åke Cederberg for help with bacteriological problems.

Mr Bengt Lindgren for revising the English text and all support.

Agneta Thomson for excellent secretarial assistance and perfect type-writing.

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Penetration of Erythromycin Through Respiratory Mucosa

A Study Using Secretory Otitis Media as a Model

BY

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Introduction

Following its introduction 25 years ago erythromycin was assigned a reserve place in the therapeutical armamentarium, as a last defence against penicillin-resistant staphylococci or as an alternative in cases of penicillin allergy. In recent years, however, erythromycin has gained a wider acceptance as a first choice antibiotic. Concomitantly, there has been an increased interest in its pharmacokinetic characteristics. The penetration of erythromycin has been studied in human semen (Eliasson et al., 1978), in bronchial secretion in chronic bronchitis (Fraschini et al., 1978), in maxillary sinus secretion in chronic sinusitis (Paavolainen et al., 1977) and in acute purulent sinusitis (Axelsson & Brorson, 1974; Kalm et al., 1975). Some aspects of the absorption of erythromycin were recently investigated by Malmborg (1978).

The respiratory mucosa consists normally of ciliated columnar cells with some goblet cells, covered by a thin layer of mucus (Tos & Bak-Pedersen, 1976). Morphologically and

physiologically the middle ear mucosa constitutes a part of the upper airway system (Flisberg, 1966; Sadé, 1966*a*; Henzer, 1970; Lim, 1974; Hagan, 1977). The respiratory mucosa reacts in a uniform manner to an inflammatory stimulus. In Secretory Otitis Media (SOM) the response of the mucosa conforms to this general pattern, which is characterized by an increased number of mucus glands and goblet cells, producing an abundance of more or less mucoid secretion (Sadé, 1966*b*; Sadé & Eliezer, 1970; Bak-Pedersen & Tos, 1971; Tos, 1976). This uniform mode of response is also seen in bronchitis and sinusitis. SOM may thus be regarded as a suitable model for the study of antibiotic penetration of respiratory mucosa, and the results should be valid for inflammatory diseases in any area lined with respiratory epithelium (Fig. 1 A-D).

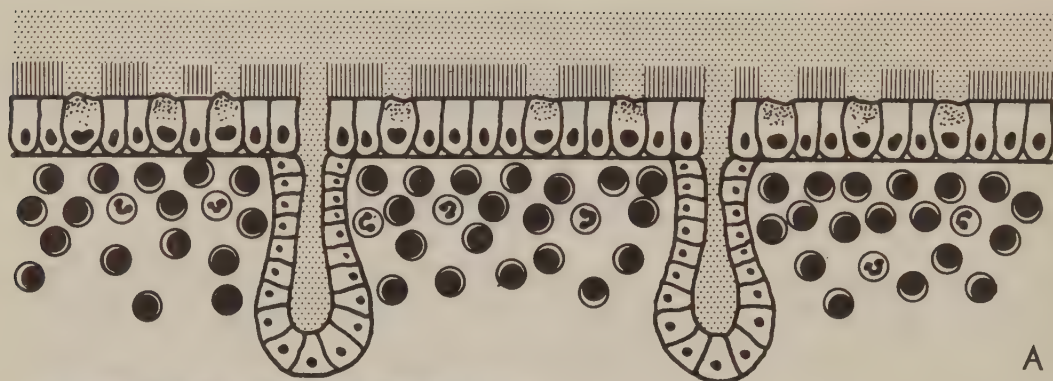
The aim of the present investigation was to study the penetration of erythromycin through respiratory mucosa, using SOM as a model.

Material and Methods

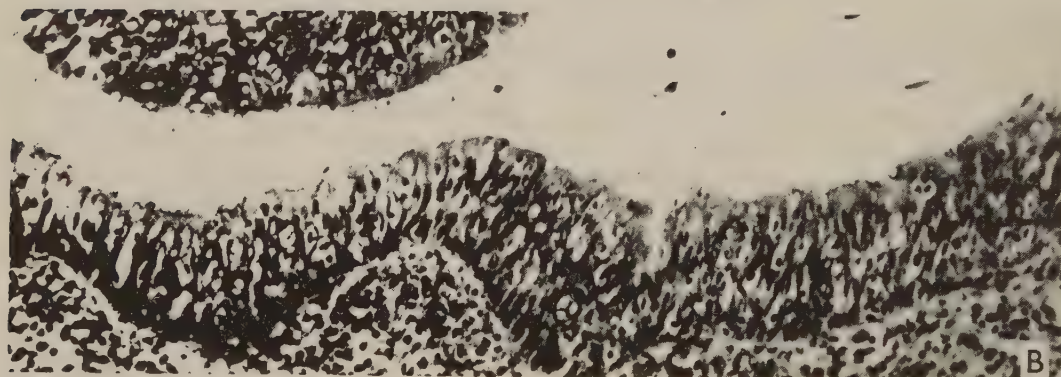
The present study is based on 108 patients with SOM who attended the ENT clinic, Central Hospital, Karlskrona. Patients participating in the investigation fulfilled the criteria of SOM: fluid behind an intact ear drum at otomicroscopy, and a non-purulent middle ear effusion at myringotomy. Only patients who did not respond to conventional therapy with decongestants were accepted for the study.

Cases of middle ear effusions caused by malignant diseases or barotrauma were excluded.

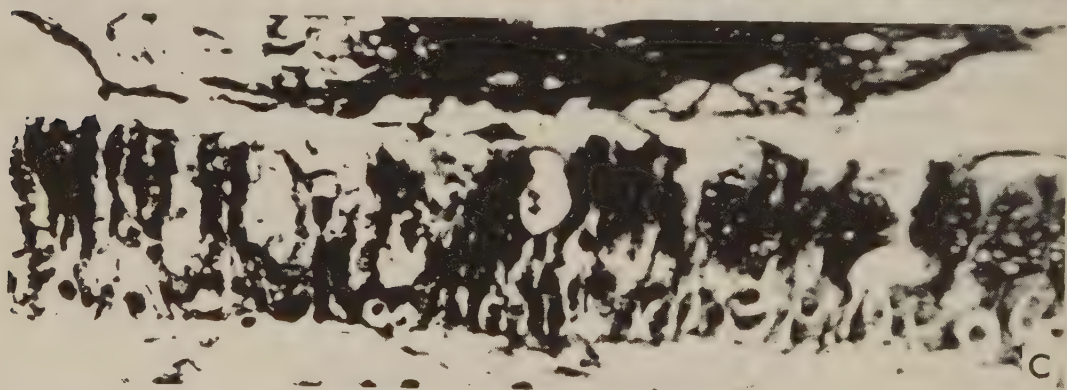
Before myringotomy erythromycinethylsuccinate (Abbotycin®) was administered orally in three doses daily (morning, noon and evening) according to recommended standard dosage (Table I) for different periods of time. The patients were divided into nine groups to



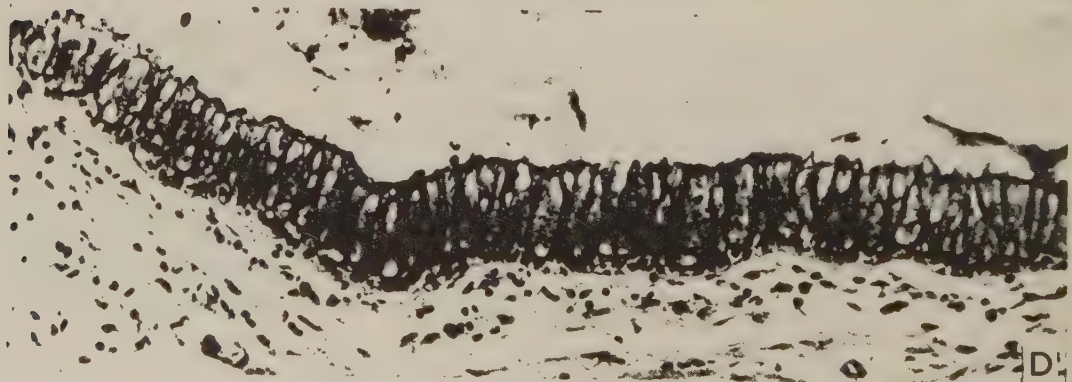
A



B



C



D

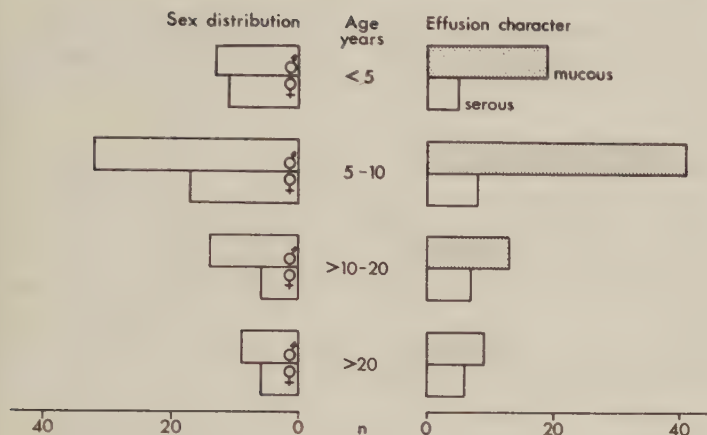


Fig. 2. Distribution according to age, sex and the character of the effusions.

investigate the early and late input phase, the middle ear steady state and the output phase. In the input phase myringotomy was performed at 2 and 14 hours (early input, after the 1st and the 2nd dose) and at 26 and 38 hours (late input, after the 4th and the 5th dose) after initiated treatment. The middle ear steady state is here represented by sampling after 2 and 10 days of administration. The output phase comprises groups where myringotomy was performed 14, 26 and 38 hours after completion of 10 days of treatment.

As a rule otomicroscopy and myringotomy was performed under general inhalation anesthesia. The median interval between the last dose and myringotomy was $2\frac{1}{2}$ hours, except when the output phase was studied. The middle ear effusion was aspirated with a sterile suction tip and collected in a sterile plastic tube, which was sealed to avoid evaporation. Specimens contaminated with blood were discarded. Blood samples for determinations of the plasma concentration were drawn simultaneously to myringotomy. The middle ear effusions were assessed according to their vis-

cosity as either serous or mucous. Fig. 2 shows the distribution according to sex, age and the character of the effusions. The majority were children with a predominance of boys. In the younger age groups (<10 years) the ratio between mucous and serous effusions was 4.6/1, in the older children and adults (>10 years) the ratio was 1.7/1.

Assay of erythromycin in plasma and middle ear secretions

An agar well diffusion method on DST agar (Oxoid), pH 7.6, was used. Plates (23×23 cm) were inoculated with *Sarcina lutea* ATCC 9341 by flooding. Wells containing about $20 \mu\text{l}$ (diameter 2.6 mm) were punched out and filled to the brim with samples. The plates were incubated at $+37^\circ\text{C}$ overnight. Two diameters at right angles to each other were measured around each diffusion centre, the mean being taken as the diameter of the inhibition zone.

Table I. Erythromycin dosage scheme

Weight kg	Daily doses g	Dose/day g	Dose/weight mg/kg
10-14	0.2+0.2+0.2	0.6	60-42
15-19	0.2+0.2+0.4	0.8	53-42
20-25	0.4+0.4+0.4	1.2	60-48
26-90	0.4+0.6+0.6	1.6	62-18

Fig. 1. (A). Schematic drawing of respiratory tract mucosa, representative of SOM, chronic sinusitis and chronic bronchitis. Shaded area indicates mucous blanket. (B) Middle ear mucosa in a case of SOM. Htx-eosin, $\times 250$. (C) Maxillary sinus mucosa in chronic sinusitis. MacManus $\times 650$. (D) Bronchial mucosa in chronic bronchitis. MacManus, $\times 210$.

Table II. Comparison between regression lines obtained by erythromycin concentrations 4.0–0.13 mg/l in plasma versus in middle ear secretions

	Experiment number										Total
	1	2	3	4	5	6	7	8	9	10	
t_{par}	1.14	0.42	0.39	0.52	0.27	0.16	1.66	0.52	0.29	0.93	
t_{id}	9.85	5.87	6.74	8.03	5.42	7.71	4.07	11.8	24.1	4.51	
Percentage concentration ^a	66±7	64±10	72±8	59±9	74±9	71±7	79±9	70±5	70±6	73±12	70±9

t_{par} : *t*-test for parallelity. All values give $p > 0.20$.
 t_{id} : *t*-test for identity. All values give $p < 0.001$.
^a $\frac{\text{Concentration calculated from erythromycin/plasma regression line}}{\text{Concentration calculated from erythromycin/middle ear secretion regression line}} \times 100$

Each sample was determined in duplicate on the same plate.

Plasma stock solution

Erythromycin U.S.P. base lot no. 48-050-CD was obtained from Abbott Laboratories, Chicago, Ill., USA, and had a certified potency of 940 mg/g. 851.05 mg of dried substance was dissolved in 100 ml methanol. Five ml of this solution was diluted to 50 ml with pooled heparinized human plasma from healthy blood donors and the pH was adjusted to 7.6. This stock solution of erythromycin in plasma containing 800 mg/l was divided into aliquots and stored at -20°C.

Standard solutions

Middle ear secretions from children with untreated SOM were collected and pooled. The effusions had too high a viscosity for serial diluting and therefore had to be treated with a 20% solution of *N*-acetyl-cysteine (Inspir®), until precision pipetting with small volumes could be carried out. The amount of *N*-acetyl-cysteine solution required was roughly 10% of the final volume. Four standard solutions of erythromycin in the concentration range 4.0–0.13 mg/l were prepared from the plasma stock solution by serial double dilutions using the following diluents: pooled middle ear secretions with roughly 2% *N*-acetyl-cysteine; plasma; plasma with 2% *N*-acetyl-cysteine; and 20% *N*-acetyl-cysteine.

Regression lines

To determine regression lines ten experiments were carried out, each consisting of testing the four standard solutions in duplicate on the same plate. The regression lines were parallel in each experiment ($0.10 < p < 0.90$). The inhibition zones produced by erythromycin in pooled middle ear secretions were significantly smaller ($p < 0.001$) than those produced by the antibiotic in the other three diluents. Between these latter no significant difference was found ($0.05 < p < 0.90$). The concentrations of erythromycin in pooled middle ear secretions, as calculated from the regression line produced by the standard solutions in plasma were thus found to be lower than the expected values. The range was 59–79%, mean 70% with a S.D. of 9% (Table II). The

Table III. Erythromycin concentrations in human plasma

Control solutions. See text

	Solution		
	I	II	III
Calculated concentration	0.4	1.6	3.2
Number of determinations	61	64	46
Mean	0.43	1.63	3.28
Range	0.33–0.56 (77–130)	1.34–2.00 (82–123)	2.40–3.92 (73–120)
S.D.	0.05 (12)	0.14 (9)	0.34 (10)

Figures within parentheses denote concentrations expressed as % of mean.

Table IV. *Erythromycin concentration in middle ear effusions*

Repeated determinations

	Sample								Total
	1	2	3	4	5	6	7	8	
<i>n</i>	6	6	8	8	6	5	6	5	50
Mean	0.29	0.49	0.60	1.14	1.12	1.70	1.44	2.32	(100)
Range	0.16–0.35 (55–121)	0.29–0.65 (59–132)	0.47–0.65 (78–125)	0.95–1.41 (83–123)	0.91–1.33 (82–119)	1.30–2.17 (76–126)	1.22–1.80 (85–125)	1.78–2.77 (77–119)	(55–132)
S.D.	0.07 (24)	0.13 (26)	0.10 (16)	0.15 (14)	0.14 (13)	0.36 (21)	0.21 (14)	0.48 (21)	(18)

Figures within parentheses denote concentrations expressed as % of mean.

experiments were performed in 3 days during which time the activity of erythromycin in the *N*-acetyl-cysteine containing solutions did not decrease.

Assay routine

Aspirated middle ear secretions and heparinized capillary blood arrived at the laboratory one day after collecting. Secretions and plasma were assayed immediately against standard solutions of erythromycin in plasma. The concentrations of erythromycin in middle ear secretions were calculated as 1.4 times the concentrations obtained from the regression line produced by the plasma standard solutions (cf. Table II).

Plasma controls

Erythromycin in plasma in concentrations of 0.4, 1.6 and 3.2 mg/l was prepared from the plasma stock solution, divided into aliquots and stored at -20°C . At least one of these con-

trols was tested on every assay plate. The results are shown in Table III.

Multiple determinations of erythromycin concentration in middle ear secretions

On eight occasions the supply of middle ear effusion was sufficient for redetermining the concentration 5–8 times. These samples were stored at $+4^{\circ}\text{C}$ and redetermined once or twice daily during the following days, until all the material was used. None of these samples were stored for more than 5 days. Table IV shows the results.

Non-specific antibacterial activity

Whenever possible what was left from the sample of middle ear secretion was added to a well in DST agar and inoculated with an erythromycin-resistant strain of *Sarcina lutea*, isolated locally. Non-specific antibacterial activity was not observed.

Results

The results are presented in Fig. 3 and Table V.

Erythromycin in middle ear effusion

In the early input phase there was a slow penetration of erythromycin into the middle ear ef-

fusion. Two hours after the initial dose the mean value was <0.13 mg/l. However, 12 hours later, i.e. about 2 hours after the second dose, the mean level was 0.6 mg/l. Not until in the late input phase, after 26 and 38 hours, did the concentration of the middle ear effu-

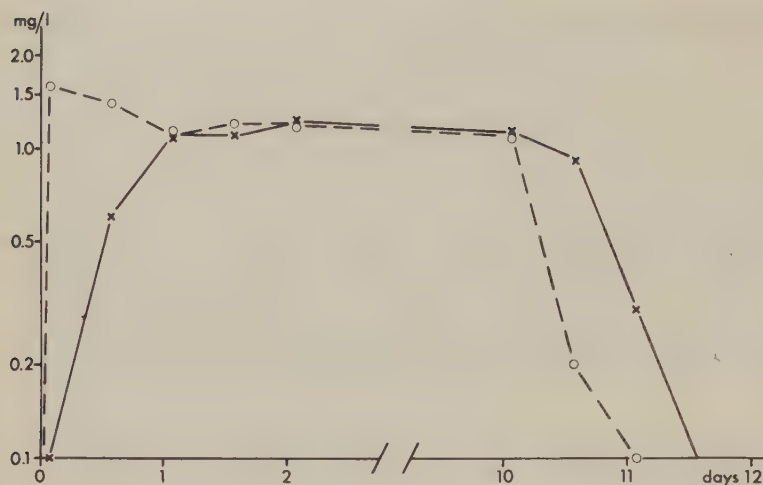


Fig. 3. Erythromycin concentration in middle ear effusion and in plasma. —: concentration in middle ear effusion; ---: concentration in plasma. All values shown are the means of 10 or more determinations except on day 11½, where there are only two cases. Shaded area denotes erythromycin therapy.

sion reach its plateau level of about 1.1 mg/l. This level was maintained during the middle ear steady state, being 1.2 mg/l on day 2 and 1.1 mg/l on day 10.

The output phase was characterized by a slow elimination. 14 hours after the last dose of 10 days' treatment, the mean middle ear effusion level was still as high as 0.9 mg/l, implying a maintained middle ear steady state. 26 hours after the last dose, however, the elimination had proceeded and the erythromycin concentration had decreased to 0.3 mg/l. Another 12 hours later there was no erythromycin

activity left in the effusion. This implies a biological half-life of about 12 hours in the secretions, should the elimination proceed rectilinearly.

Erythromycin in plasma

Two hours after the initial dose the mean plasma level was 1.6 mg/l and 12 hours later, i.e. 2 hours after the second dose, it was 1.4 mg/l. The mean plasma levels were then maintained at 1.1–1.2 mg/l up to the 10th day. In the output phase there was a rapid elimination. 14

Table V. A survey of the results of the entire series

	Early input		Late input		Middle ear steady state		Output phase		
	2 h	12 h	24 h	36 h	48 h	10 d	12 h	24 h	36 h
<i>Plasma</i>									
Mean mg/l	1.6	1.4	1.1	1.2	1.2	1.1	0.2	0.1	0.0
S.D.	0.69	1.1	0.5	0.6	0.9	0.8	0.2	0.1	
Range	0.5–3.0	0.5–4.2	0.6–2.0	0.2–1.8	0.4–3.2	0.0–4.2	0.6–0.4	0.0–0.3	
n	10	10	10	11	14	27	12	12	2
<i>Ear eff.</i>									
Mean mg/l	0.1	0.6	1.1	1.1	1.2	1.1	0.9	0.3	0.0
S.D.		0.5	0.7	0.5	0.6	0.9	0.7	0.3	
Range	0.0–0.4	0.3–1.5	0.3–2.2	0.2–2.3	0.4–2.7	0.4–4.2	0.0–2.5	0.0–0.8	
n	10	11	13	16	17	27	12	17	2
<i>Cultures</i>									
n	8	10	10	7	8	27	12	8	2

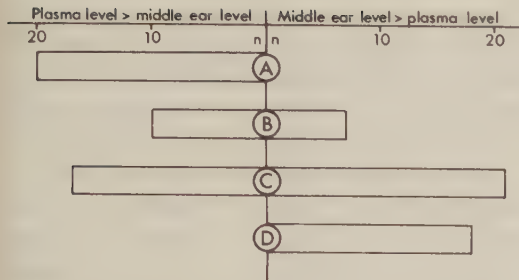


Fig. 4. A graphical demonstration of the course of penetration. (A) Early input phase; (B) late input phase; (C) middle ear steady state; and (D) output phase. Cases with identical concentration in plasma and middle ear effusion are not included.

hours after the last dose of 10 days' treatment the mean plasma level was down to 0.16 mg/l, and a further 12 hours later it was <0.13 mg/l. It is generally known that the plasma half-life of erythromycin is in the order of 2 hours (e.g. Malmberg, 1978)—in sharp contrast to the estimated half-life in the middle ear effusions, which was in the magnitude of 12 hours.

General aspects

Fig. 4 displays graphically the course of the penetration. In the early input phase the individual determinations showed a higher concentration in plasma than in the effusion. In the late input phase and at the middle ear steady state there was a balance in this respect. The output phase was a mirror image of the input phase; here the middle ear effusions contained more erythromycin than plasma.

According to recommended standard erythromycin dosage, children are given 40–60 mg/kg body weight/day whereas adults receive less. Table VI demonstrates the results of

Table VI. The age distribution of the erythromycin concentration in plasma 2 hours after the last dose ($n=82$) and in middle ear effusion on the 2nd and 10th day ($n=41$)

Observe that despite different dosages (mg/kg) the mean levels are similar

	Age group, years			
	<5	5–10	>10–20	>20
<i>Plasma</i>				
<i>M</i> , mg/l	1.3	1.2	1.0	1.3
<i>S.D.</i>	0.9	0.8	0.7	1.1
<i>n</i>	22	33	16	11
<i>Ear eff.</i>				
<i>M</i> , mg/l	1.1	1.0	1.4	1.1
<i>S.D.</i>	0.5	0.8	1.3	
<i>n</i>	11	18	9	3

plasma concentration and effusion levels at middle ear steady state in different age groups. There was no significant difference despite the dissimilarity in dosage.

As there was only a small number of serous secretions the material does not allow a comparison of the penetration of erythromycin into secretions of different viscosities. However, the values obtained in serous secretions were in good agreement with those in mucous.

It has been stated that middle ear secretions (Siirala & Lahikainen, 1952), like sinusitis secretions (Kalm et al., 1975) have an antibacterial effect of their own. Such an antibacterial effect could not be demonstrated in our material.

In 17 cases samples were assessed from both ears. The results tallied very well—identical in 9 cases and in 7 cases there was a difference of less than 20% between the pair of ears. In only one case was there a marked dissimilarity.

Discussion

It should be pointed out already at the beginning that the present investigation was not undertaken to propose antibiotic treatment of

Secretory Otitis Media (SOM)—conventional treatment including the use of grommets is still an unsurpassed mode of therapy. However,

SOM is in our opinion a very suitable model for studying antibiotic penetration of respiratory mucosa (Sundberg et al., 1978).

In recent years interest in the penetration of antibiotics has increased considerably. There has been a lack of knowledge of the penetration characteristics, even as regards antibiotics that have been in use for several decades, such as erythromycin. The reason for this may be the earlier view that the plasma concentration was the only salient parameter. It has become increasingly evident that the concentration of an antibiotic in plasma does not give sufficient information about its ability to penetrate into the tissues. Taken to the extreme, it is not unlikely that a very high plasma concentration of an antibiotic implies that it is confined to the blood and therefore penetrates the tissues to a smaller extent than may be surmised (Norrby, 1978). In modern pharmacokinetics, attention has shifted to the concentration of the antibiotic in the very focus of inflammation.

Penetration studies may be performed in several different ways. Interstitial fluid concentrations can be determined by inert chamber (e.g. Holm et al., 1978; Norrby et al., 1978; Chisholm, 1978) or skin blister methods (e.g. Simon, 1976; Schreiner et al., 1978). Direct determination of tissue concentrations is another useful technique, although blood contamination is an inherent source of error that may influence the results. The numerous samples needed to gain a general understanding of the penetration process is another obstacle in methods applied directly to tissues, especially in areas such as the middle ear where biopsies cannot be performed indiscriminately. Studies of secretions are commonly performed and offer many advantages. Normal and pathological secretions are often available in sufficient amounts to enable determinations. It is now generally accepted that antibiotic concentration in a secretion is always lower than in its mucosa (Eneroth, 1976; Eneroth & Lundberg, 1976). Adequate concentration in the effusion implies more than

adequate levels in the mucous membranes. Albeit determination of antibiotic penetration into secretion from the middle ear is an indirect way, it still affords an estimation of the mucous membrane concentration. Thus studies based on secretion are informative without loss of reliability and relevance.

SOM is often seen as a non-purulent continuation of a purulent otitis media. In other cases the debut is insidious, most often in connection with an upper respiratory infection. In its initial stage there is an increased number of goblet cells and a development of mucus glands in the epithelium (Tos & Bak-Pedersen, 1973*a, b*; Sadé, 1974; Tos & Bak-Pedersen, 1977). The second stage is characterized by an accumulation of mucus in the middle ear to such an extent that the clearing action of the mucociliary system is overloaded. This stage has a long duration of several months or more. Thus there is a long period during which sampling can be performed with such a uniform case material that inter-individual differences may be considered very small. Eventually the disease enters the stage when mucus glands degenerate, the epithelium returns to its normal state and resolution takes place.

SOM has been the subject of many studies, most of them focused on therapy and, in recent years, on etiology. The penetration of antibiotics has been investigated in only a few instances. The use of SOM as a model for the study of antibiotic penetration on the respiratory mucosa has not been proposed earlier.

Our choice of SOM for penetration studies is based upon the following considerations. The middle ear mucosa is a part of the upper respiratory tract—embryologically, morphologically and physiologically. Its histological picture conforms to the general pattern of respiratory mucosa, with ciliated columnar epithelium intermingled with goblet cells (Bak-Pedersen & Tos, 1973). In inflammatory diseases the middle ear mucosa reacts in the same way as other parts of the upper respiratory tract mucosa, with a considerable increase in the number of goblet cells and development of

mucus glands, both producing a large amount of mucus. Therefore the results of penetration studies into the middle ear effusion would be valid not only for the middle ear itself but for the whole upper respiratory tract.

Earlier reports on the penetration of antibiotics have mainly focused on the input phase, fewer have dealt with later stages of the course, and the output phase is generally disregarded. For a comprehensive examination of penetration, all three aspects should be clarified. Consequently many samples have to be obtained during the whole period of antibiotic treatment. In clinical practice this often presents difficulties as, on one hand, the number of individual cases must be large and, on the other, the material must be as uniform as possible.

In cases of SOM which do not respond to conservative therapy, myringotomy with insertion of a grommet is the standard procedure. The middle ear effusion does not recur as long as the grommet is functioning. Therefore, in using SOM as a model for penetration studies we are confined to a single sample of secretion per case. However, SOM is a very common condition and the clinical picture is singularly uniform, as is the histological appearance of the mucosa. At grommet insertion the middle ear effusion is always aspirated and samples are thus obtained without extra procedures. Furthermore, contamination with blood seldom occurs and is easily detected in the otomicroscope. Moreover, the samples are taken under aseptic conditions.

Ideally, the erythromycin concentration in middle ear effusions should be assayed by means of a set of concentration standards in the same type of secretion. The amount of middle ear effusion that could be obtained for standard solutions was not sufficient for the large number of determinations required in this study. Furthermore the viscous middle ear secretions had to be pretreated in order to reduce their viscosity and *N*-acetyl-cysteine was used for this purpose. Diffusion of erythromycin from these pretreated SOM secre-

tions was slower than from plasma, giving smaller zones of inhibition. Evidently this was not due to the presence of *N*-acetyl-cysteine, since the same amounts of this substance in plasma did not influence the activity of erythromycin in our regression line experiments. Moreover, the reduction of the zone diameters was of the same magnitude as observed by Kalm et al. (1975) when they compared the diffusion from sinus secretions and serum, using essentially the same technique.

In our experiments the regression lines from middle ear secretions and plasma were not identical but parallel, permitting us to calculate the erythromycin concentration in the middle ear effusions from the plasma concentration standard lines with a correction factor of 1.4 (Table II). The assay of erythromycin was less accurate for SOM secretion samples (Table IV) than for plasma (Table III), mostly due to difficulty in filling the wells right to the brim with the sometimes very viscous material. The accuracy was nevertheless considered adequate for the purpose of this study. The good agreement of the results when samples from both ears of the same patient were determined supports this opinion.

There are only a few previous studies on antibiotic penetration into the middle ear effusion in cases of SOM. Silverstein et al. (1966) reported nine cases of SOM where five were given penicillin and four received oxytetracycline i.m. in a single dose. Sampling was performed 1–3 hours later. There were only traces of oxytetracycline in the middle ear secretions; penicillin penetrated in four cases but in small amounts. Lahikainen (1970) studied the penicillin concentration in 22 cases of SOM, one and 2–4 hours after a single i.m. dose. There were traces of penicillin in the effusions in only nine cases, mostly at 2–4 hours after the administration. Lahikainen et al. (1977) compared the penetration of azidocillin and ampicillin, given in a single standard dose. 74 samples were obtained from 63 patients at 1, 2, 8 and 12 hours after oral administration. Both penicillins penetrated

slowly and only low levels were found. Klimek et al. (1977) studied the concentration of amoxicillin and ampicillin 1–2 hours after a fourfold standard single oral dose in 28 cases of SOM. Amoxicillin penetrated four times better than ampicillin. Thus earlier studies on antibiotic penetration in cases of SOM have all been performed on smaller series, have merely comprised the early input phase and all of them have been single-dose procedures. Erythromycin has not been investigated at all.

In the present investigation the penetration of erythromycin through the respiratory mucosa in cases of SOM was followed and elucidated during the whole course—early and late input phase, middle ear steady state and output phase. Our results show that erythromycin penetrates into the middle ear secretion, albeit with a slow input phase. Highest middle ear values were obtained 24 hours later in the ear than in plasma. In the output phase the clearance of erythromycin from the middle ear secretion was also slow. The elimination was not completed until more than 24 hours after the last dose given. In the middle ear steady state the concentration of erythromycin was the same in middle ear effusions as in concomitantly drawn plasma samples. It should be observed that the plasma levels were invariably determined about 2 hours after the last dose given—except in the output phase—and that they therefore represent approximately plasma peak levels. Thus the concentration of erythromycin in the middle ear effusion reaches a level which corresponds to the plasma peak level. It is a moot question whether erythromycin penetrates merely by diffusion or if there is also a mechanism for active secretion.

In the output phase the mean concentration of erythromycin in the middle ear secretion 12 hours after the last dose was still as high as 0.9 mg/l, having only decreased from 1.1 to 0.9 mg/l. At the same time the plasma level was as low as 0.16 mg/l—a decrease from 1.1 to 0.16 mg/l. These facts show that the middle ear effusion has the ability to retain erythro-

mycin for a considerable time. Malmborg (1978) has shown that even after 7 days of oral erythromycin treatment three times daily the plasma levels still vary considerably, the peak level being about five times higher than the lowest value. The plasma half-life is about 2 hours. Therefore, when during interdose intervals the plasma level decreases below the peak level, the erythromycin concentration in the middle ear effusion remains essentially unchanged at the plasma peak value. Thus erythromycin not only penetrates the respiratory mucosa in the middle ear to attain levels in the secretion equivalent to plasma peak levels, but it is also retained in the middle ear effusion for a relatively long period of time. This fact allows us to use the term 'steady state' for the middle ear secretion.

The calculated half-life in the effusion during the output phase was about 12 hours. This should be compared with a plasma half-life of about 2 hours. Now, there are no specialized structures for resorptive purposes in the middle ear, nor is there any evidence supporting the idea that erythromycin might be metabolized in the effusion. An antibiotic that is trapped in the middle ear secretion can be eliminated only through two routes: by mucociliary clearance via the eustachian tube and by rediffusion into plasma. As erythromycin is retained in the secretion the output phase apparently reflects a slow mucociliary clearance. In SOM there exists a state of equilibrium between the production of secretion and its elimination via the eustachian tube. The turnover rate is still unknown (Sadé, 1967). According to the discussion above one may expect a turnover rate of not less than 12 hours. In this context it would be interesting to study whether there is any difference in the input–output characteristics between mucous and serous secretions, possibly reflecting differences in mucociliary activity. The highly viscous mucus ought to impede mucociliary clearance more than the serous secretion (Sadé et al., 1975). However, our series comprises too few cases with serous secretions to

allow us to draw any conclusions on this aspect.

A contributing factor to the slow penetration and elimination phases in SOM may be the fact that the antibiotic has to pass into and out from an essentially closed cavity filled with a more or less mucoid secretion. Though the mucosa shows all the signs of an inflammatory process there is nevertheless a pronounced difference in degree between the vascular response in SOM and in acute purulent otitis media. Thus the general condition in SOM may in itself constitute a partial physiological barrier for antibiotic penetration. More far-fetched explanations for a prolonged elimination may be based upon the hypothesis that erythromycin has a special affinity for some constituent in the effusion or has advantageous properties of solubility in it. It is still a matter for speculation how other antibiotics would penetrate the respiratory mucosa in SOM. The macrolides and the tetracyclines are partially soluble in lipids, whereas the penicillins are not. The field is open for further investigations.

As a rule bacterial inflammations of the upper respiratory tract display a shorter acute purulent phase superseded by a longer mucus-productive stage. The inflammatory response of the mucosa in SOM represents this later stage of an acute inflammation. In fact, there is overwhelming circumstantial evidence for the inflammatory pathogenesis of SOM—such as the histological appearance of the mucosa (Bernstein & Hayes, 1971; Lim & Birck, 1971; Lundgren & Rundcrantz, 1976; Sadé & Weissman, 1977) and the presence of high levels of immunoglobulins and lysozymes in the effusion (Liu et al., 1975; Palva et al., 1976; Juhn et al., 1977). Many studies have demonstrated the presence of aerobic bacteria in middle ear effusions in an incidence that varies from 22% to 77% (Senturia et al., 1958; Healy & Teele, 1977; Palva et al., 1978). Bacterial cultures of aural effusions yielded several strains of bacteria, most frequently *Staphylococcus epidermidis*, diphtheroid species, *Haemophilus influenzae* and various strains of streptococci.

It is well known that the penetration of antibiotics is enhanced during the acute purulent phase. However, what happens to the penetration of antibiotics when the inflammation changes to the more protracted mucoid stage? Lundgren & Rundcrantz (1976) followed the penetration of penicillin into the middle ear secretion in acute otitis media during a 6-day course of therapy. During the purulent stage, the first day, ample concentrations of penicillin were obtained, being about half the serum level. However, later in the course of the disease, when the secretion had turned mucoid in appearance and similar to that in SOM, very much lower levels of penicillin were found in the middle ear effusions, about 10% of the concentration in serum. Thus penicillin readily passed into the ear secretion in the acute phase—the first day. From the second day on its penetration decreased considerably as the disease turned into a stage similar to SOM. There is a similar observation in cases of acute sinusitis. Lundberg & Malmberg (1973) found in the initial stage a good penetration of penicillin. On the 4th to 5th day, when the secretion had turned mucoid and SOM-like, low concentration of penicillin was obtained, less than 20% of plasma peak levels. Tetracycline, on the other hand, attained concentrations in the mucoid sinusitis secretion that were equal to and even sometimes surpassed the peak plasma levels.

There is a striking parallelism between the penetration of tetracycline in cases of sinusitis and erythromycin in cases of SOM. Both antibiotics readily passed into these mucus-filled bone-enclosed cavities lined with respiratory mucosa, and both antibiotics there attained concentrations equal to plasma peak levels. It should be observed that it is during the later, mucoid stage of the inflammation that the scales are tipped—either the mucosa returns to normalcy or it becomes irreversibly damaged, the disease process turning into a chronic condition. A suitable antibiotic that penetrates this mucoid stage may well turn the balance in favour of resolution.

There are only a few studies on the penetration of erythromycin in other localities of the respiratory tract. Axelsson & Brorson (1974) used erythromycin estolate, 1 g daily orally for 10 days, and found in the secretion of acute maxillary sinusitis a mean concentration of 1.8 mg/l 6 hours after the last dose. Kalm et al. (1975) studied 20 patients with acute sinusitis, half were given 1.5 g erythromycin stearate daily, the other half received 1.0 g daily. Sinus secretions were obtained on the 3rd and 5th day about 4 hours after the last dose and the mean concentration of erythromycin in the first group was 1.3 mg/l, in the second 0.6 mg/l. Eleven samples were purulent, nine were

mucous. Paavolainen et al. (1977) studied 15 patients with chronic maxillary sinusitis, using erythromycin stearate 1.5 g daily orally. The average concentration in the secretion after 4 days of treatment was 1.2 mg/l and in the mucosa 1.8 mg/l. The last dose was given 2 hours before sampling. The penetration of erythromycin stearate into bronchial secretions in cases of chronic bronchitis was determined by Fraschini et al. (1978). They found a mean concentration of 1.0 mg/l. These reports support our concept that antibiotic penetration of the respiratory mucosa is largely independent of its location.

Conclusion

Secretory otitis media has been shown to be an appropriate model for penetration studies, the results of which are applicable to the entire upper respiratory tract. Erythromycin penetrated into the middle ear effusion and from 26 hours the mean concentration of erythromycin

in the effusion was equal to the mean plasma peak level. The output phase in the secretion was considerably prolonged compared with the rapid elimination of erythromycin from plasma.

Summary

This study is based upon the concept that the respiratory mucosa reacts in a uniform manner to an inflammatory stimulus. Secretory Otitis Media (SOM) may be used as a model to disclose some aspects of antibiotic penetration. Erythromycin was given for different periods of time to 108 cases of SOM where myringotomy was indicated. The middle ear effusion was aspirated and blood samples were obtained simultaneously. The concentration of erythromycin was determined by microbiological procedures.

Erythromycin penetrated into the middle ear effusion. After the fourth dose the concentration was at the same level as the plasma peak level. The elimination of the drug from the middle ear secretion was considerably prolonged compared with the rapid elimination from plasma. This implies that erythromycin attains a steady state in the middle ear effusion with concentrations equal to the plasma peak level.

Zusammenfassung

Die vorliegende Studie basiert auf dem Konzept, dass die respiratorische Schleimhaut

gleichartig auf einen infektiösen Reiz reagiert, und somit die seröse Otitis media als Modell

zur Aufschlüsselung einiger Aspekte der Antibiotikapenetration dienen kann. In 108 Fällen von seröser Otitis media mit Indikation zur Myringotomie wurde Erythromycin in verschiedenen Zeitintervallen präoperativ verabreicht. Die Erythromycinkonzentrationen im Aspirat der Paukenhöhle sowie in den simultan entnommenen Blutproben wurden durch mikrobiologische Methoden ermittelt.

Es zeigte sich, dass Erythromycin in Pau-

kenerguss bereits nach der vierten Dosis das Niveau der maximalen Plasmakonzentration erreichte. Im Vergleich zur schnellen Ausscheidung aus dem Plasma ist die Ausscheidung aus dem Mittelohrsekret bedeutend verlängert. Es ist daher anzunehmen, dass Erythromycin im Paukenerguss eine 'steady-state' Konzentration erreicht, die der maximalen Plasmakonzentration entspricht.

Résumé

Cette étude est basée sur la notion que la muqueuse respiratoire, réagissant d'une manière uniforme à un stimulus inflammatoire, on peut se servir de l'Otite Sécréteuse Médiane (SOM) comme modèle pour exposer certains aspects de pénétration des antibiotiques. Dans 108 cas de SOM où la myringotomie était indiquée, la décharge de l'oreille médiane fut aspirée et la quantité d'érythromycin fut déterminée par procédés microbiologiques. Des analyses de sang furent obtenues par la même occasion. Après la quat-

rième dose la pénétration d'érythromycin dans la décharge de l'oreille médiane, atteint déjà des concentrations égales au niveau maximum du plasma.

En comparaison l'élimination d'érythromycin de l'oreille médiane est considérablement plus prolongée que l'élimination du plasma. Cela implique que l'érythromycin atteigne les concentrations stables (*steady-state*) de l'oreille médiane égales au niveau maximum dans le plasma.

Resumen

Este estudio se basa en que la mucosa respiratoria reacciona de una manera uniforme a un estímulo inflamatorio. La Otitis Serosa Media (SOM), puede servir de modelo para exponer ciertos aspectos de la penetración de los antibióticos. En 108 casos de SOM en donde la myringotomía estaba indicada, la secreción del oído medio fué aspirada y la cantidad de eritromicina se determinó por procedimientos microbiológicos. Al mismo tiempo, análisis de sangre fueron hechos. Después de la cuarta

dosis, la penetración de eritromicina en la secreción del oído medio alcanzó ya concentraciones iguales al nivel máximo en el plasma.

En comparación, la eliminación de eritromicina del oído medio es considerablemente mas prolongada que la eliminación del plasma. Esto significa que la eritromicina alcanza las concentraciones estables (*steady-state*) del oído medio iguales al nivel máximo en el plasma.

Acknowledgements

The authors are greatly indebted to Professor Anders Bergstrand, Sabbatsbergs sjukhus, and

Professor Nils Jonsson, Lunds lasarett, for the histologic pictures. Abbott Scandinavia AB

provided financial support. The translation was revised by David Allen, M.D., the French summary was translated by Mme Chantal Husberg and the Spanish summary was translated

by Jaime Alvarez de Ibarguren, M.D. Mrs Barbro Nordanfors is thanked for secretarial assistance.

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ACTA OTO-LARYNGOLOGICA

Supplement 384: 3-9

1981

II

PENETRATION OF ERYTHROMYCIN IN WALDEYER'S RING - ADENOID TISSUE

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PENETRATION OF ERYTHROMYCIN IN WALDEYER'S RING—ADENOID TISSUE

L. Sundberg, T. Edén and S. Ernstson

Abstract. Erythromycin was given orally to 72 children with adenoid enlargement. Adenoidectomy was performed at different times during treatment. The concentration of erythromycin in adenoid tissue homogenate, in blood and in plasma was determined according to a microbiologic agar-well diffusion method. Blood-free tissue levels were calculated. Already after the first dose the concentration of erythromycin in tissue reached the same level as the concomitant plasma concentration. During the course of treatment the tissue concentration was maintained at a therapeutic level. After the last dose a rapid elimination took place. The tissue concentration in the adenoid was ten times above the MIC of *S. pneumoniae* and *Br. catarrhalis*, but not for *H. influenzae*.

In today's ENT practice relapsing otitis media in younger children is a commonly encountered problem with considerable social and economic implications. The nasopharynx and the adenoid play an important role in respiratory tract infections by offering a suitable environment for bacteria. Respiratory pathogens may survive in this locality even during a course of apparently adequate antibiotic treatment. *Streptococcus pneumoniae* for example, though extremely sensitive to betalactam antibiotics, often persists in the nasopharynx of children with acute otitis media after several days of treatment with penicillin V (Kamme et al., 1970). After cessation of therapy the surviving bacteria may reinvade the middle ear to start a new episode of otitis media. This relapse often occurs within one month of the onset of the primary infection and is thus more a continuation or a reactivation of the initial infection than a new otitis media (Kamme, 1971).

Bacterial persistence might depend on an insufficient penetration of antibiotics to the nasopharynx with its adenoid (Nylén, 1975). However, a comprehensive study on antibiotic penetration to the adenoid has not yet been

published. The purpose of the present investigation was to study the penetration of erythromycin to adenoid tissue.

MATERIAL AND METHOD

This investigation is based on the participation of 72 otherwise healthy children that showed such enlargement of the adenoid that adenoidectomy was indicated. The parents had given their informed consent before the children entered the study. The age and sex distribution of the children is presented in Table I. The operations were performed during infection-free intervals and antibiotics had not been given for at least two weeks prior to the present.

Erythromycin ethylsuccinate (Abbotcin®, Abbott) was given orally twice a day (morning and evening) in standard dosage (Table II) for different periods of time before adenoidectomy (Table III). The children were divided into six groups according to the duration of treatment. All doses but the last one were given with food in order to improve resorption (Malmberg, 1978) and to decrease the frequency of gastro-intestinal disturbances. In group 6 the last dose, 14 hours before surgery, was also taken with food.

Adenoidectomy was performed under general anaesthesia according to standard procedure and blood samples were drawn simultaneously. Adenoid tissue, whole blood and plasma were collected and the concentration of erythromycin was assessed.

Laboratory procedure

The adenoid tissue samples were cut to small pieces and homogenized mechanically with an

Table I. Age and sex distribution

Years	<5	5-10	>10
Male	10	21	2
Female	13	23	3

Ultraturrax® tissue grinder. The tissue homogenate, whole blood and plasma were stored frozen at -70°C until analyzed. Three sets of erythromycin standard solutions were prepared in the concentration range 0.13–4.0 mg/l: the plasma standard (pooled normal human heparinized plasma); the whole blood standard (pooled normal human heparinized freeze-thawed blood) and the tissue standard (pooled homogenates of adenoids taken from patients that had not received antibiotic).

The agar-well diffusion method used in this assay has been described in detail previously (Sundberg et al., 1979). The regression lines obtained by the whole blood standard and the tissue standard turned out to be identical. Erythromycin concentrations in adenoid tissue homogenates were therefore calculated from the whole blood standard regression line in the assay. Control solutions of erythromycin in plasma, in whole blood and in tissue homogenate were tested on every assay plate. The mean values differed from the calculated concentrations by 3–5 %. The SDs were 8–13 % of the means. Haemoglobin concentrations in blood and in adenoid tissue homogenate were determined by standard methods.

The assessment of erythromycin in whole blood and in homogenized tissue together with haemoglobin determination enabled calculation of the concentration of erythromycin in blood-free tissue. The following formula was evolved:

$$C_t = \frac{C_s - C_b \cdot k}{1 - k}$$

C_t , C_s and C_b denote respectively erythromycin concentration in blood-free tissue, in sample (= homogenized tissue) and in whole blood. The coefficient of blood contamination (k) is

Table II. Oral erythromycin ethylsuccinate dosage

Body weight (kg)	Dosage (g)	Dose/weight (mg/kg)
15–19	0.4×2	53–42
20–25	0.6×2	60–48
26–58	0.8×2	61–28

given by the ratio between haemoglobin in tissue homogenate and in whole blood. The derivation is described in detail in Appendix. Thus, it should be observed that in this investigation all tissue concentrations are given as blood-free levels.

RESULTS

The results are displayed in Fig. 1 (mean values) and in Table IV. Erythromycin penetrated the adenoid tissue rapidly—already three hours after the first dose a mean tissue level of 1.3 mg/l was reached, i.e. a level almost identical to the concomitant plasma concentration. Later in the course the mean tissue levels 2 to 3 hours after the second to fifth doses ranged between 0.9–1.3 mg/l. The corresponding mean plasma levels were 0.8–0.9 mg/l. The mean tissue concentrations of erythromycin were thus similar to or even somewhat higher than the concomitant plasma levels, although the difference was not significant. After the last dose a rapid elimination occurred—the

Table III. Duration of therapy before adenoidectomy and sampling

Group	No. of patients	Duration of therapy (h)	No. of doses	Hours between last dose and operation (mean $\pm$ SD)
1	12	1–3	1	1.9 ± 0.8
2	12	12	2	2.6 ± 0.5
3	11	24	3	2.6 ± 0.6
4	15	36	4	2.7 ± 0.7
5	12	48	5	3.1 ± 0.9
6	10	48	5	14.0 ± 0.8

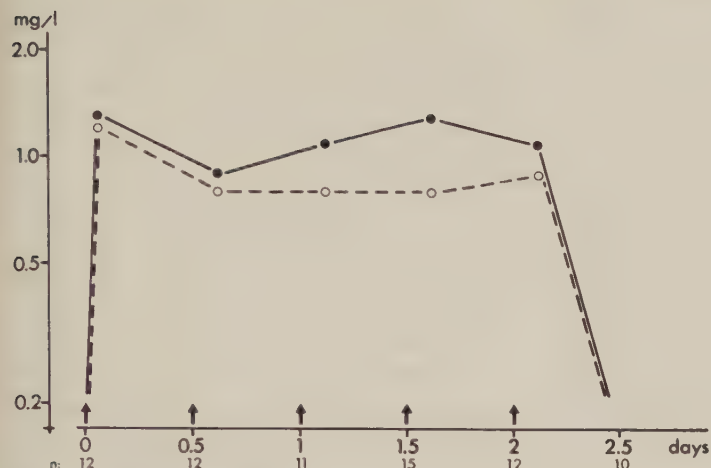


Fig. 1. Erythromycin penetration into adenoid tissue, mean values and corrected against blood contamination. The continuous line shows the tissue levels, the broken

line shows the plasma values. Arrows indicate doses, *n*, number of cases in each group.

mean tissue and plasma levels 14 hours later were below 0.2 mg/l.

The haemoglobin determinations showed a mean tissue-to-blood haemoglobin ratio of 0.06 (range 0.02–0.27). The blood contamination of adenoid tissue homogenates thus turned out to be in the order of 6%, which tallies with other estimates (Ekedahl et al., 1978).

DISCUSSION

Although many studies deal with the penetration of antibiotics to the middle ear and the

paranasal sinuses (e.g. Silverstein et al., 1966; Kamme et al., 1969; Lundberg & Malmberg, 1973; Axelsson & Brorson, 1974; Carenfelt et al., 1975; Lahikainen et al., 1977; Paavolainen et al., 1977; Ekedahl et al., 1978; Klimek et al., 1979; Sundberg et al., 1979; Ingvarsson et al., 1980), there are few investigations on the penetration of antibiotics to the adenoid (Gaillard et al., 1971; Panzer et al., 1972; Sundén & Schiratzki, 1977). This is the more surprising as the nasopharynx with its adenoid is recognized as an important source of infection in otitis media and in sinusitis, especially in children.

Table IV. A survey of the results of the entire series

	Input					Output 12 h
	3 h	12 h	24 h	36 h	48 h	
Plasma						
Mean, mg/l	1.2	0.8	0.8	0.8	0.9	0.2
SD	0.7	0.9	0.4	0.6	0.6	
Range	0.4–2.4	0.1–3.6	0.1–1.6	0.2–2.3	0.1–2.2	0.0–0.6
Adenoid tissue						
Mean, mg/l	1.3	0.9	1.1	1.3	1.1	0.1
SD	1.1	0.8	0.9	0.9	0.7	
Range	0.2–3.7	0.2–2.4	0.2–3.4	0.4–3.5	0.2–2.7	0.0–0.4
<i>n</i>	12	12	11	15	12	10

The bacterial colonization of the nasopharynx in children takes place particularly in the secretions covering the adenoid (Lundberg & Lönnroth, 1979). The environment in this ecological niche seems to offer suitable conditions for respiratory pathogens such as *S. pneumoniae*, *Haemophilus influenzae*, *Branhamella catarrhalis* and β -haemolytic streptococci group A. Thus, in cases of acute otitis media in children nasopharyngeal cultures yielded respiratory pathogens in almost 100% (Howie & Ploussard, 1971; Kamme, 1971), and among otitis-prone children a pathologic nasopharyngeal flora was obtained in 76%, even though there were no signs of respiratory tract infection at the time of sampling (Nylén, 1975). In cases of secretory otitis media the frequency of respiratory pathogens isolated from the nasopharynx was as high as 84% (Ingvarsson et al., cit. Lundgren & Rundcrantz, 1976) and 79% (Sundberg et al., this supplement).

Acute otitis media nowadays heals spontaneously in the majority of cases (Thomsen et al., 1980). Nevertheless, in individual cases the disease shows a recurrent nature, especially during the first years of life (Howie et al., 1975). It is notable that these relapses occur despite a seemingly adequate penicillin therapy. Bacterial persistence in the nasopharynx has been demonstrated in many of these cases (Kamme, 1971; Lundgren, 1972).

Thus, in children with acute otitis media treated with an appropriate dosage of penicillin V for 10 days, *S. pneumoniae* persisted in the nasopharynx in 30–40% and *H. influenzae* in 73% (Kamme et al., 1970; Herberts et al., 1971). In another investigation where cases of acute otitis media were treated with either azidocillin or ampicillin, a short temporary decrease in the number of nasopharyngeal strains of *S. pneumoniae* occurred, while no influence was exerted on *H. influenzae* (Frölund Thomsen et al., 1975).

In an attempt to reduce the relapses several different schemes for sequential treatment with antibiotics have been tried (Björklund et al., pers. comm.; Thomsen et al., 1980) or

suggested (Kamme et al., 1978), but the results have not yet been encouraging. Such treatment could only be expected to be successful if an elimination of the respiratory pathogens is achieved in the middle ear and in the nasopharynx with its adenoid. In avoiding relapses, it is important, therefore, to use antibiotics that penetrate adequately the secretions in the middle ear and the nasopharynx including the adenoid tissue. Studies on antibiotics with such penetration characteristics are rare and concern antibiotics that for other reasons are unsuitable in the treatment of acute otitis media, such as spiramycin (Gallard et al., 1971), clindamycin (Panzer et al., 1972) and penicillin G (Sundén & Schiratzki, 1977).

In the present investigation different aspects of the penetration of erythromycin to the adenoid tissue are elucidated. Erythromycin belongs to the group of antibiotics that passively diffuses into tissues and secretions, the concentration gradient deciding the rate and degree of penetration. Under such circumstances it is evident that the peak concentration must always be higher in the tissue than in the secretion but below the peak level in plasma. The results showing a mean tissue concentration in the order of 1.3–1.4 mg/l are in full agreement with this pharmacokinetical law. In an earlier study (Sundberg et al., 1979) the corresponding mean value in respiratory secretions, represented by middle ear secretions in cases of secretory otitis media, was found to be 1.1 mg/l. Since the respiratory mucosa lining the adenoid is similar to that in secretory otitis media, a similar level of erythromycin can be inferred in the mucous secretion covering the adenoid.

In sum: erythromycin ethylsuccinate in this dosage brings about a plasma peak level of 1.5–3.5 mg/l, a mean adenoid tissue level of 1.3–1.4 mg/l and a mean respiratory tract secretion level of 1.1 mg/l (Fig. 2).

S. pneumoniae and *Br. catarrhalis* are very sensitive to erythromycin with very low MICs in vitro (Kislak et al., 1965; Finland et al.,

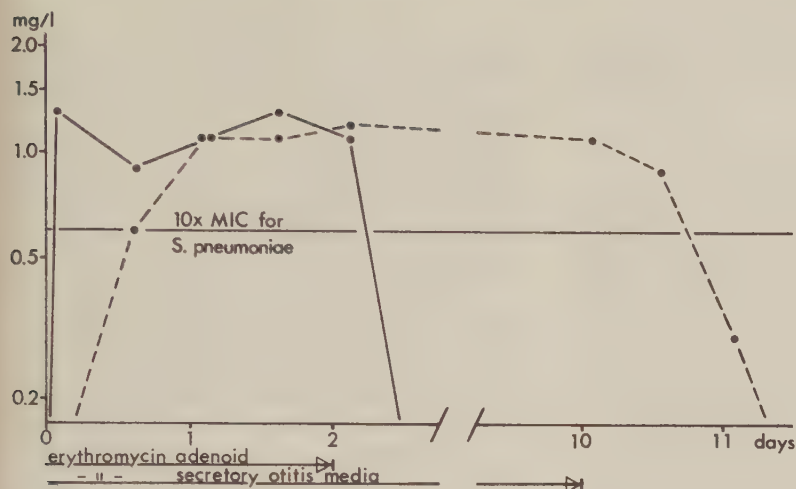


Fig. 2. Erythromycin penetration of adenoid tissue and respiratory tract secretion. A composite graph showing the results of two studies, the present (adenoid tissue, continuous line) and a previous (secretory otitis media,

broken line) (Sundberg et al., 1979). Observe the close correspondence in the respective levels, and the rapid penetration into tissue in contrast to the slower penetration of the middle ear secretion.

1976; Cleary et al., 1978). Our studies have thus shown that the mean levels of erythromycin obtained in the adenoid tissue and in the secretions covering it are ten times above the MICs of these common respiratory pathogens (Fig. 2). On the other hand, most strains of *H. influenzae* do not possess a similar susceptibility to erythromycin, the MICs in vitro ranging 0.5–25 mg/l (Gould, 1977). Consequently, the concentration of erythromycin in the adenoid tissue will often be insufficient to eradicate *H. influenzae*.

Clinical investigations dealing with bacterial persistence in the nasopharynx during erythromycin therapy have not yet been presented. If respiratory pathogens, especially *S. pneumoniae*, should survive, like they do during penicillin therapy, different possibilities should be taken into consideration. As can be seen in Table IV, the range of the concentration values in plasma and in tissue is very wide—a feature common to most penetration studies. The resorption of the drug is individual, and some very low concentrations are found in a few patients. Bacterial persistence could be pre-

sumed to occur more readily in these patients than in general. This, however, is a hypothesis that remains to be proven. Nevertheless, in clinical practice it might be advisable to monitor the absorption of the antibiotic in cases of therapeutic failures.

It should be observed that phenomena like bacterial tolerance (Lundberg, 1977; Acar & Sabath, 1978), L-forms and beta-lactamase-producing commensals (Brook, 1981), often discussed in connection with bacterial persistence during treatment with penicillin V, do not apply to erythromycin.

Recently polyvalent pneumococcal vaccines have been given to children in an attempt to reduce the number of relapses (Kafma et al., 1980). Unfortunately, children respond very weakly to antigenic stimuli during the first years of life. For the time being antibiotics with satisfactory penetration characteristics in combination with adequate antibacterial activity seem to be a better alternative.

In conclusion. Erythromycin has been shown to penetrate the middle ear and the respiratory mucosa (Sundberg et al., 1979) and

the adenoid tissue. The levels obtained should be sufficient to eradicate most respiratory pathogens.

ZUSAMMENFASSUNG

An 72 Kindern mit Rachenmandelhyperplasie wurde Erythromycin oral verabreicht. In verschiedenen Intervallen nach Beginn der Antibiotikabehandlung wurde eine Adenotomie durchgeführt. Die Erythromycinkonzentration im Gewebehomogenisat aus der Rachenmandel, Vollblut und Plasma wurde nach einer mikrobiologischen Agar-Gel Diffusionsmethode bestimmt. Der Gewebespiegel des Erythromycins wurde aus den oben genannten Werten errechnet. Schon nach der ersten Verabreichung war der Gewebespiegel gleich der im Plasma erreichten Konzentration. Im Laufe der Behandlung wurde ein therapeutisches Niveau der Gewebekonzentration aufrechterhalten. Nach der letzten Verabreichung wurde Erythromycin rasch ausgeschieden. In der Rachenmandel wurde ein Gewebespiegel erreicht der dem zehnfachen MHK-wert für *S. pneumoniae* und *Br. catarrhalis* entsprach, jedoch nicht für *H. influenzae*.

RÉSUMÉ

De l'érythromycine fut donnée oralement à 72 enfants atteints de végétations adénoïdes. L'ablation des végétations fut pratiquée à différents moments pendant le traitement. La concentration d'érythromycine dans le tissu adénoïde après homogénéisation, dans le sang et le plasma, fut déterminée selon une méthode de diffusion microbiologique avec "l'agar-well" méthodique. On compta les niveaux de tissus cellulaires dépourvus de sang. Déjà après la première dose, la concentration d'érythromycine dans les tissus atteignit le même niveau que celui du plasma concomitant. Pendant la durée du traitement, la concentration dans le tissu cellulaire se maintient à un niveau thérapeutique. Après la dernière dose eut lieu une rapide élimination. La concentration dans le tissu adénoïde était dix fois au dessus du niveau MIC de *S. pneumoniae* et *Br. catarrhalis*, mais pas pour *H. influenzae*.

RESUMEN

Eritromicina se dió por vía oral, a 72 niños con hiperplasia adenoidea. Adenoidectomía se practicó a diferentes tiempos durante el tratamiento. La concentración de eritromicina en el tejido adenoideo homogeneizado, en la sangre y en el plasma, fué determinada de acuerdo con el método microbiológico de difusión con agar-well técnica. El tejido libre de sangre fué calculado. Después de la primera dosis, la concentración de eritromicina en el tejido adenoideo alcanzó el mismo nivel que la concentración en el plasma. Durante el curso del tratamiento, la concentración en el tejido adenoideo se mantuvo a nivel terapéutico. Después de la última dosis, una rápida eliminación se produjo. La concentración en el tejido adenoideo fué diez veces mas alta que el MIC del *S. pneumoniae* y *Br. catarrhalis*, pero no del *H. influenzae*.

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APPENDIX

Hb_b : Blood haemoglobin

k : Coefficient of blood contamination in sample

Hb_s : Sample haemoglobin

$$(1) \quad k = \frac{Hb_s}{Hb_b}$$

V_s : Total volume of sample

V_b : Volume of blood in sample

V_t : Net volume of tissue

$$(2) \quad V_b = V_s k$$

$$(3) \quad V_t = V_s - V_b$$

$$(4) \quad V_t = V_s - V_s k; \quad V_t = V_s(1-k)$$

C_s : Drug concentration in sample

D_s : Total amount of drug in sample

C_b : Drug concentration in blood

D_b : Total amount of drug in blood

$$(5) \quad D_s = V_s C_s$$

$$(6) \quad D_b = V_b C_b$$

Substituting (2) in (6) gives us

$$(7) \quad D_b = V_s C_b k$$

D_t : Total amount of drug in tissue

C_t : Drug concentration in tissue

Substituting (5) and (7) in (8) gives

$$(8) \quad D_t = D_s - D_b$$

$$(9) \quad D_t = V_s C_s - V_s C_b k$$

$$D_t = V_s(C_s - C_b k)$$

$$C_t = \frac{D_t}{V_t}$$

$$C_t = \frac{V_s (C_s - C_b k)}{V_s (1-k)}$$

$$C_t = \frac{C_s - C_b k}{1-k}$$

ACTA OTO-LARYNGOLOGICA
Supplement 384: 10-17
1981

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PENETRATION OF ERYTHROMYCIN IN WALDEYER'S RING - TONSIL TISSUE

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PENETRATION OF ERYTHROMYCIN IN WALDEYER'S RING—TONSIL TISSUE

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Abstract. This study is based on the participation of 26 otherwise healthy patients with a diagnosis of chronic tonsillitis. Erythromycin ethylsuccinate (Erythrocin®, Abbott) 1 000 mg was given orally twice daily to 20 cases and tonsillectomy was performed after different intervals. Erythromycin was assessed in the homogenized tonsils, in plasma and in blood. Haemoglobin determinations in blood and tissue homogenate enabled calculating blood-free tissue levels. Remarkably, the tissue levels were in the order of twice the plasma levels. The remaining 6 patients received the erythromycin mixture via gastric tube, to prevent surface contamination of the tonsils. In these cases the tonsil tissue levels were the same as the plasma levels. Erythromycin thus penetrates tonsil tissue to attain the same levels as in plasma. A corollary of the adenoid and tonsil tissue penetration studies is that erythromycin may be relied upon to penetrate any lymphatic tissue to the same degree.

Concomitant to the advent of antibiotics, the panorama of infectious diseases changed completely. Antibiotics enabled us to cope with acute ENT-infections in a way that forty ears ago was wishful thinking only. However, the infectious diseases have also lost much of their severity. Fulminant infections are rare in today's ENT practice, and the life-threatening complications of old seem in the process of receding into the pages of medical history.

Whatever the cause or causes of this loss of severity, we are nowadays in a situation where the use of antibiotics in ENT practice has turned into over-use, possibly even abuse. For instance, Diamant & Diamant (1974) have shown that 88% of patients with acute otitis media never need antibiotics. In an impressive series of investigations, Axelsson et al. (1970, 1971, 1973, 1974, 1975 and 1981) showed that cases of acute maxillary sinusitis are best treated with irrigation and decongestive nose drops—adding any of a large number of antibiotics did not change the outcome percep-

tively. Recently, Meistrup-Larsen et al. (1980) have arrived at conclusions identical to those of Diamant & Diamant.

Nevertheless, when antibiotics are to be used, on old or new indications, there are some fundamental properties that should be known to the physician. Penetration is one of these. There is now an enormous wealth of clinical experience concerning those antibiotics that have stood the test of time: erythromycin, penicillin and tetracycline. Paradoxically enough, our knowledge about their pharmacokinetics is still in some aspects less than that of the newer products.

Several investigations have disclosed a remarkable disparity in the antibiotic penetration of tonsil tissue (Table II). The present work was undertaken to study the penetration of erythromycin into tonsil tissue and to elucidate some sources of error.

MATERIAL AND METHODS

In this study 26 patients participated, having given their informed consent. They were hospitalized under the diagnosis of chronic tonsillitis, otherwise healthy and had not taken antibiotics for at least two weeks. Their median age was 17 years (range 7-37 years) and their median weight was 51 kg (range 27-83 kg). There were 22 women and 4 men. Erythromycin ethylsuccinate (Erythrocin®) 1 000 mg was dissolved in 100 ml water and given twice a day. The median daily dosage was 39 mg/kg body weight (range 74-24 mg/kg). Tonsillectomy was performed à froid under general anaesthesia. The tonsils were removed in the usual manner and venous blood samples

were drawn simultaneously. The tonsils, one sample of blood and one sample of plasma were collected and the concentration of erythromycin was assessed according to the description below.

Twenty patients participated in the first part of the study, where erythromycin was administered per os, the last dose about three hours before surgery. In 6 cases tonsillectomy was performed after the first dose, in 4 cases after two doses, in 9 cases after three doses and in 1 case after five doses.

In a second part of the study 6 patients received their erythromycin via a gastric tube, instead of swallowing it freely, between 3 and 5 hours before surgery. In these cases the absorption was monitored by frequent plasma analyses.

Laboratory procedure

The tonsils were cut to small pieces and homogenized mechanically with an Ultraturrax® tissue grinder. The tissue homogenate, blood and plasma were stored frozen at -70°C until analyzed. Three sets of erythromycin standard solutions were prepared in the concentration range 0.13–4.0 mg/l: the plasma standard (pooled normal human heparinized plasma); the blood standard (pooled normal human heparinized freeze-thawed blood) and the tissue standard (pooled homogenates of tonsils taken from patients that had not received antibiotic).

The agar-well diffusion method used in the assay has been described in detail previously (Sundberg et al., 1979). The regression lines obtained by the blood standard and the tissue standard turned out to be identical. The concentration of erythromycin in tonsil homogenates were therefore calculated from the blood standard regression line in the assay. Control solutions of erythromycin in plasma, in blood and in tissue homogenate were tested on every assay plate. The mean values differed from the calculated concentrations by 3–5%. The SDs were 8–13% of the means. Haemoglobin concentrations in blood and tonsil

homogenate were determined by standard methods.

The determination of erythromycin and haemoglobin in homogenized tissue and in blood enabled us to calculate the concentration of erythromycin in blood-free tissue. The following formula was evolved:

$$C_t = \frac{C_s - C_b \cdot k}{1 - k}$$

where C_t , C_s and C_b denote respectively concentration in blood-free tissue, in sample (= homogenized tissue) and in blood. The coefficient of blood contamination (k) is given by the ratio between haemoglobin in homogenate and in whole blood. The derivation of the formula is described in detail in Appendix. Thus, it should be observed that in this investigation all tissue concentrations are given as blood-free levels.

RESULTS

In the first part of the study, where the patients took the erythromycin solution per os, the mean tonsil homogenate concentrations were in the order of twice the mean plasma concentrations (Fig. 1, the individual results are presented in Table I). Sampling was performed $2\frac{1}{2}$ to $4\frac{1}{2}$ hours after the administration of the last dose of erythromycin. The plasma concentrations were therefore well below the peak plasma levels, as these are obtained about one hour after oral administration (Malmberg, 1978). The differences between the tonsil homogenate concentrations and the plasma levels were plotted against the time between administration and sampling (Fig. 2). It is evident that the tonsil homogenate-to-plasma quotient ($\approx 200\%$) persisted unchanged throughout the phase of elimination.

An artifact was to be suspected and in the second part of the study 6 patients received the erythromycin solution via a gastric tube. The individual results are presented in Table I

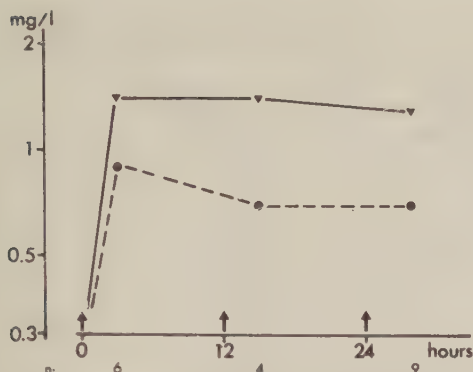


Fig. 1. Erythromycin penetration into tonsil tissue—artefactual values. The erythromycin solution was given per os and the high tonsil tissue levels relative the plasma levels show evidence of tonsil surface contamination at swallowing. The continuous line denotes erythromycin concentration in blood-free tonsil tissue homogenate; the broken line shows the erythromycin level in plasma. Arrows indicate the 1st, 2nd and 3rd dose of 1000 mg erythromycin. The values are means and *n* shows the number of cases in each group.

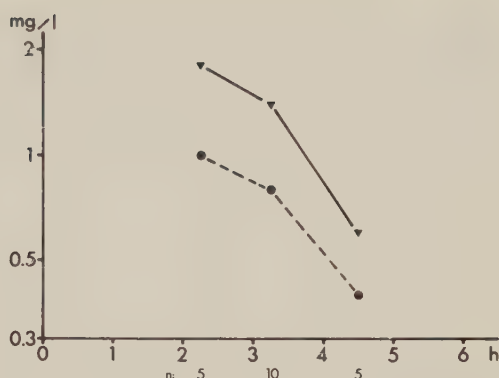


Fig. 2. Erythromycin penetration into tonsil tissue—artefactual values. The material has been divided in three groups where sampling was performed with a median interval of 2½, 3½ and 4½ hours after the last dose. Note the high tonsil tissue levels, twice the plasma levels, at all three occasions during elimination. The continuous line denotes erythromycin concentration in blood-free tonsil tissue homogenate; the broken line shows the erythromycin level in plasma. The values are means and *n* shows the number of cases in each group.

and, graphically together with the absorption curves, in Fig. 3. Though the material is small, it is nevertheless obvious that the tonsil tissue

concentrations were similar to the actual plasma levels, i.e. a tonsil-to-plasma ratio of ≈100 %. This relation was unaffected by the differ-

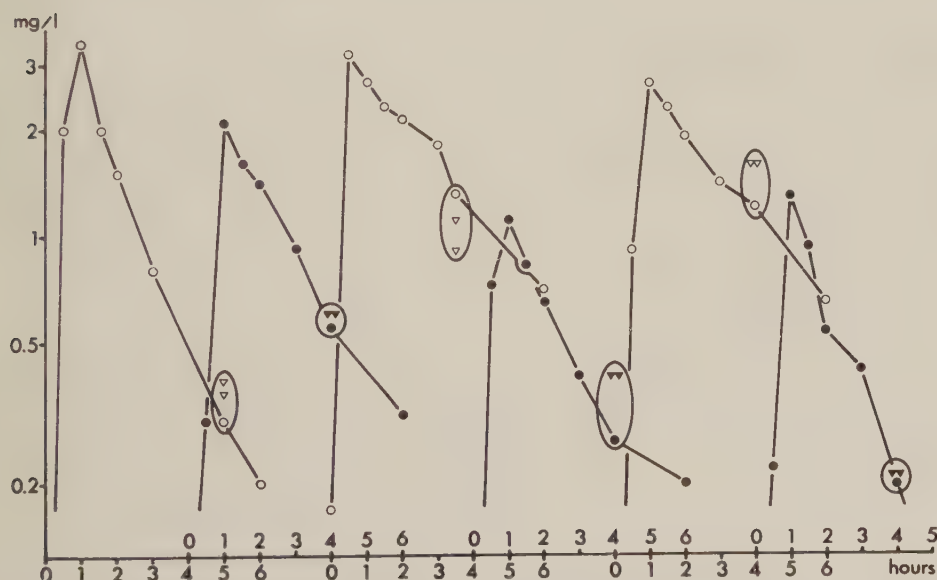


Fig. 3. Erythromycin penetration into tonsil tissue. The composite graph shows the absorption (plasma levels) in 6 patients that received 1000 mg erythromycin via gastric tube. The plasma levels and the corresponding blood-free

tonsil tissue levels are shown inside each oval. Circles, plasma levels; triangles, tonsil tissue levels. The symbols of alternate patients are filled for reasons of clarity.

Table I. A survey of the results

Patient no.	Doses no.	Route	Plasma mg/l	Tonsil A mg/l	Tonsil B mg/l	Tonsil/plasma ratio %	Time after given dose
1	1	Oral	0.2	0.6	0.6		4.0
2	1	Oral	0.3	0.2	0.2		3.8
3	1	Oral	0.8	2.0	2.0		2.5
4	1	Oral	0.9	1.5	1.4		3.0
5	1	Oral	1.2	1.6	1.6		3.5
6	1	Oral	1.7 M: 0.9	2.7	2.7 M: 1.4	155	2.7 M: 3.25
7	2	Oral	0.2	0.6	0.6		3.3
8	2	Oral	0.5	0.9	0.9		2.8
9	2	Oral	1.0	2.8	2.7		2.8
10	2	Oral	1.1 M: 0.7	1.3	1.3 M: 1.4	200	2.3 M: 2.80
11	3	Oral	0.2	0.6	0.5		3.8
12	3	Oral	0.3	1.0	1.0		2.5
13	3	Oral	0.5	0.6	0.6		5.0
14	3	Oral	0.5	1.0	0.8		3.9
15	3	Oral	0.6	1.3	1.3		3.4
16	3	Oral	0.7	1.3	1.3		3.5
17	3	Oral	1.0	1.6	1.6		3.3
18	3	Oral	1.3	2.0	2.0		3.1
19	3	Oral	1.5 M: 0.7	2.4	2.2 M: 1.3	186	3.5 M: 3.56
20	5	Oral	0.4	0.6	0.6		4.7
1	1	Via tube	0.2	0.2	0.2		4.3
2	1	Via tube	0.3	0.4	0.4		3.9
3	1	Via tube	0.3	0.4	0.4		4.9
4	1	Via tube	0.6	0.6	0.6		4.0
5	1	Via tube	1.2	1.6	1.6		3.8
6	1	Via tube	1.3 M: 0.7	1.1	0.9 M: 0.7	100	3.3 M: 4.03

ent durations between administration and sampling and by individual differences in absorption.

There was very little difference between the two tonsils. The concentration of erythromycin was the same in both tonsils in 20 cases. The difference between the two tonsils of a pair was in three cases 0.1 mg/l and in the remaining three cases 0.2 mg/l (Table I).

The haemoglobin determinations showed a mean tissue-to-blood haemoglobin ratio of 0.09 (range 0.02–0.19). The blood contamination of the tonsil tissue homogenates thus turned out to be in the order of 10%.

DISCUSSION

Tonsil tissue penetration studies are all performed in a similar manner. The material regularly consists of patients that are to be tonsillectomied, which means that most of

them are young adults. Albeit the indications for tonsillectomy may vary from one clinic to another, there is reason to assume that the spectrum of tonsil disease is similar in all studies. Accordingly, one may presume the results to be corresponding, especially when the same antibiotic is used, if given in comparable dosage and if sampling is performed after similar intervals.

Despite the fundamental similarities there are a number of variable parameters. The galenic properties of the antibiotic, the dosage route and scheme, the number of doses and the interval between administration and operation/sampling, all these and other factors co-operate to form a caleidoscopic picture from which it is impossible to construct a single true measure of antibiotic penetration. Yet, some kind of measure is a necessity to compare the results of different investigations.

Irrespective of these variations, the plasma

Table II. *Survey of literature*

Author	Antibiotic	Ad- mini- stra- tion	Tissue- to- plasma ratio %
Ginsburg et al. (1976)	Erythromycin	Orally	13-64
Kaplan et al. (1974)	PcV	Orally	20
Gould (1976, 1977)	Erythromycin	Orally	38
	PcV	Orally	160
	Erythromycin	Orally	275
Pravčev et al. (1978)	PcV	Orally	2
	Erythromycin	Orally	54
Miniti et al. (1970)	Erythromycin	Orally	94-125
	Doxycycline	Orally	61-83
Georgiew et al. (1978)	Erythromycin	Orally	190
	Oleandomycin	Orally	330
Roos et al. (1980)	PcV	Orally	22
	PcV	Orally	34-45
Beckmann et al. (1975)	PcV	Orally	92
Emolieva et al. (1969)	PcV	Orally	92
Squinazi et al. (1979)	Minocycline	Orally	160
Panzer et al. (1972)	Clindamycin	Orally	217
Bo et al. (1969)	Tetracycline	Orally	109
	Oleandomycin	Orally	114
	PcV	Orally	4-18
Chilla & Haubrich (1976)	PcV/PcG	i.m.	3-12
	PcG	i.m.	2-20
Jokinen & Raunio (1975)	Azidocillin	i.m.	26
Bernasconi (1973)	Thiamphenicol	i.m.	43-134
Brusis et al. (1977)	Azidocillin	i.v.	24
Gabka & Platz (1978)	PcG	i.v.	7-65
	Ampicillin	i.v.	19-42
	Lincomycin	i.v.	72-113
For the sake of completeness the following references are listed, where the plasma/serum levels are not reported:			
Berrettini (1968)	Ampicillin and Doxycycline		
Gaillard et al. (1971)	Spiramycine		
Galetti et al. (1978)	Ampicillin		

concentration curves (the absorption curves) are largely similar in their configuration. With few exceptions, the tissue concentrations are dependent on the plasma levels. A frequently used measure of tissue penetration is therefore given by the ratio between the tissue level and the concomitant plasma level. This measure allows some degree of comparison between different antibiotics and different studies, and is largely independent of different plasma elimination times.

The divergent results of tonsil tissue pene-

tration studies are displayed in Table II. The tissue-to-plasma concentration ratios vary from 2 to 160 % for the penicillins; from 13 to 275 % for different preparations of erythromycin and may reach 330 % for odd antibiotics such as oleandomycin. A low tissue-to-plasma ratio is note-worthy but not uncommon. The opposite, i.e. a high tissue level notwithstanding a normal or even low plasma level is more seldom. In these instances special care should be taken to rule out artifacts.

An obvious methodological aspect when concentrations are assessed by microbiological means is that the standard solutions for determinations of tissue concentration should be made on homogenized tissue, to give the antibiotic the same environment in standards as in samples. Kaplan et al. (1973) showed that if this procedure is not attended to, an artifact occurs rendering the penicillin values too low and giving too high erythromycin levels. Even though the method is not always described in detail, it is probable that most authors have circumvented this fallacy, like we have.

Blood contamination almost invariably entails artifactual results. If the blood level differs from the real tissue level the uncorrected tissue concentration values will be distorted one way or another. In our study the blood contamination was found to be small, in the order of 10 %, and the ratio between tonsil and blood concentration favourable, about 100 %. Thus, in the present investigation the error would have been small even if the tissue concentrations had remained uncorrected. Larger errors are to be expected when the antibiotic level in the blood is high relative to the true tissue level.

The use of the tissue-to-plasma ratio as a measure of antibiotic penetration entails erroneous results if small numbers of samples are taken and if the drug in the tissues is not at distribution equilibrium with plasma. In this investigation these obstacles were supposed to be overcome by studying a fairly large number of patients in different groups, each group representing different phases of a normal

course of erythromycin therapy, in complete accordance with earlier investigations (Sundberg et al., 1979; *idem*, this suppl.). However, the disparity between the tonsil tissue homogenate levels and the concomitant plasma levels, as observed when the medication had been swallowed—the normal mode of administration—forced us to abandon the original program and to search for a tenable explanation. A trapping phenomenon, where erythromycin unimpeded would penetrate the tonsil tissue, there to be restrained from re-entering the circulation, did not seem plausible.

In the latter part of the study the drug was given via a gastric tube. In this way all erythromycin found in the tonsil tissue must have been brought there via the blood. Although the material is small, it was nevertheless obvious that the tonsil tissue concentrations were similar to the concomitant plasma levels, i.e. the tissue-to-plasma ratio was in the order of 100%.

Our results thus disclose a third artifact and one that is a feature particular to tonsil tissue determinations, viz. that the antibiotic may be entrapped in the tonsil crypts or adhere to the tonsil surface when the medication is swallowed. This artifact would occur most frequently in those investigations where the antibiotic is given as a fluid, less often when it is given in tablets and not at all when it is administered via capsules, parenterally or, as in the latter part of our study, via a gastric tube. The amount of erythromycin imbibed by the tonsils via the "external" route was about the same as that carried via the blood. The surface contamination was effective for a considerable time, as is evident from Fig. 2.

Kaplan et al. (1974) and Ginsburg et al. (1976) administered antibiotics in the form of syrup and found the tonsil tissue levels to be only 13–64% of the plasma levels. On the other hand, Gould (1976, 1977), also using solutions, reported erythromycin and penicillin to penetrate to respectively 275 and 160% of the concomitant plasma levels.

Our results showing an erythromycin penetration of tonsil tissue equal to the plasma levels tallies with the results from a parallel investigation on adenoid tissue (Sundberg et al., this supplement). The adenoids and the tonsils are, apart from different epithelial linings, morphologically identical. Surface contamination at swallowing could not occur in the adenoid study, and here the tissue levels also followed the plasma levels closely during the whole course of erythromycin therapy. From the fact that adenoids and tonsils are composed mainly of lymphatic tissue it may be inferred that erythromycin penetrates other lymphatic tissues to the same degree.

It should be observed that the plasma concentrations shown in the first part of this study do not represent peak levels. In the 6 patients where peak levels were determined (Fig. 3), these were in accordance with previous investigations, i.e. between 1.5 and 3.5 mg/l (Malmberg, 1978).

As erythromycin penetrates the tissue of chronic tonsillitis to attain the same concentrations as in plasma, it may be relied upon to penetrate no less readily in acute tonsillitis. The extra amount of erythromycin that is imbibed in the tonsils "externally" when the suspension is swallowed should not be counted upon to partake in the antibacterial effort in acute tonsillitis. Regardless of the mode of administration, erythromycin upholds its place in the therapeutic arsenal—the results from clinical experience are unequivocal in this matter. Tonsil and adenoid tissues are rapidly penetrated and the tissue levels are equal to the concomitant plasma levels.

ZUSAMMENFASSUNG

Dieser Arbeit liegt ein Material von 26 Patienten zugrunde, die abgesehen von einer chronischen Tonsillitis gesund waren. In 20 Fällen wurden 1000 mg Erythromycin Äthylsuccinat (Erythrocin®, Abbott) zweimal täglich oral verabreicht, und in unterschiedlichen zeitlichen Abständen wurde eine Tonsillektomie durchgeführt. Daraufhin wurde das Homogenisat aus Tonsillen, sowie Plasma und Blut auf die Erythromycinkonzentration hin untersucht. Durch die Hämoglobinbestimmung im Voll-

blut und Tonsillenhomogenisat wurde das Errechnen der Gewebekonzentration ermöglicht. Dabei fiel auf dass der Erythromycinspiegel im Gewebe in der Grössenordnung des zweifachen Plasmawertes lag.

Die hohe Gewebekonzentration lässt sich durch die perorale Kontamination der Tonsillen erklären. Die restlichen 6 Patienten erhielten daher Erythromycin durch eine Magensonde. Bei diesen Patienten wurde eine Gewebekonzentration in den Tonsillen gemessen die der aktuellen Plasmakonzentration entsprach. Dieses bedeutet, dass die Erythromycinpenetration in Tonsillengewebe so gut ist, dass eine gleichmässige Verteilung zwischen Plasma und Gewebe erreicht wird. Es ist anzunehmen, dass die Penetration in andere lymphatische Gewebe gleichermaßen gut ist.

RÉSUMÉ

Cette étude est basée sur la participation de 26 patients bien portants mais avec le diagnostic de tonsillite chronique. Dans 20 cas, de l'érythromycine éthylsuccinate (Erythrocin®, Abbott) 1000 mg leur fut administrée oralement deux fois par jour et l'ablation des amygdales fut pratiquée après différents intervals. La quantité d'érythromycine fut évaluée dans les amygdales homogénéisées, dans le plasma et dans le sang. Les déterminations d'hémoglobine dans le sang et le tissu homogène permet de calculer les niveaux des tissus dépourvus de sang. Remarquablement, les niveaux des tissus étaient deux fois plus élevés que les niveaux du plasma. Dans les six cas restants, on administra de la mixture d'érythromycine par le tube gastrique, pour éviter la contamination en surface des amygdales. Dans ces cas les niveaux du tissu tonsillaire étaient les mêmes que ceux du plasma.

L'érythromycine pénètre ainsi les tissus tonsillaires et atteint les mêmes niveaux que dans le plasma. Un corollaire des études de la pénétration des tissus adénoïdes et tonsillaires est que l'on peut compter sur l'érythromycine quand il s'agit de pénétrer n'importe quel tissu lymphatique au même degré.

RESUMEN

Este estudio se basa en 26 enfermos con el diagnóstico de tonsilitis crónica, por lo demás sanos. Un gramo de etilsuccinato de eritromicina (Erythrocin®, Abbott) fué administrado por vía oral dos veces al día, a 20 enfermos y una tonsilectomía se practicó a diferentes intervalos. La concentración de eritromicina fué determinada en el tejido tonsilar homogeneizado, en el plasma y en la sangre. La determinación de hemoglobina en la sangre y en el tejido tonsilar homogeneizado, hizo posible el cálculo de la cantidad de tejido libre de sangre. Remarcablemente, la concentración de eritromicina en el tejido tonsilar fué aproximadamente dos veces mas alta que en el plasma.

A los 6 enfermos restantes, se les administró eritromicina en forma líquida utilizando una sonda gástrica para evitar la contaminación de las tonsilas. En estos seis casos, la concentración de eritromicina en el tejido tonsilar fué la misma que en el plasma. La eritromicina ha

por lo tanto penetrado en el tejido tonsilar hasta alcanzar el mismo nivel que en el plasma. Una conclusión de estos estudios de la penetración de eritromicina en los tejidos adenoideo y tonsilar, es que la eritromicina puede penetrar de la misma manera cualquier tejido linfático.

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ACTA OTO-LARYNGOLOGICA

Supplement 384: 18-25

1981

IV

BACTERIOLOGY IN SECRETORY OTITIS MEDIA

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BACTERIOLOGY IN SECRETORY OTITIS MEDIA

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Abstract. Nasopharyngeal cultures from children with longstanding Secretory Otitis Media (SOM), obtained under general anaesthesia, disclosed colonization of respiratory pathogens in 79% of the cases, though there was no evidence for recent infection in the ENT region. In a partly overlapping series of cultures from the middle ear effusions the same strains of respiratory pathogens were isolated in 18% of the cases. The pathogens, *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Branhamella catarrhalis*, appeared as single species or in various combinations. The occurrence of beta-lactamase producing strains may inactivate betalactam antibiotics. There is cause to believe that an antibiotic with special characteristics should be looked for, to be used on strictly chosen indications in an attempt to increase the resolution of SOM.

Secretory otitis media (SOM) is an enigmatic disease whose etiology, true incidence and best mode of treatment have eluded otologists for more than a century. During the last decennia there has been a marked increased interest in SOM and several aspects have been brought to light. For instance, the middle ear mucosa in SOM, a respiratory tract mucosa, has shown changes consistent with an inflammatory etiology (e.g. Senturia, 1970; Tos, 1976; Juhn et al., 1977; Lewis et al., 1979; Sadé, 1979; Tos et al., 1979) and several species of pathogens have been isolated in the middle ear secretions (e.g. Kokko, 1974; Liu et al., 1975; Healy & Teele, 1977; Giebink et al., 1979; Lim et al., 1979). Analyses of the effusions have at times indicated an allergic etiology (e.g. Draper, 1967; Veltri & Sprinkle, 1973; Palva et al., 1978; Palva, 1980).

In current opinion there are two main etiological factors. On one hand an inflammatory process, less virulent than the acute otitis media but continuing for a far longer time; on the other hand a reaction probably immunological by nature and mediated by bacterial

antigens from some remote locality (Bernstein & Ogra, 1980; Carenfelt, 1980; Palva et al., 1980). SOM is thus a disease of its own, not by necessity a complication to or an attenuated continuation of an acute otitis media.

The nasopharynx occupies a central position in these matters. Respiratory pathogens are found there in 15–20% of normal children (Norstedt, 1967; Nylén, 1975). During acute otitis media nasopharyngeal cultures yield pathogens in almost 100% with a close correlation to the flora in the middle ear (Howie & Ploussard, 1971; Kamme, 1971). Ingvarsson et al. (cf. Lundgren & Rundcrantz, 1976) found pathogens in the nasopharynx in 84% of 96 cases of SOM, and Laurell et al. (1980) arrived at an identical figure.

The present investigation was undertaken to study the bacterial flora in the nasopharynx and in the middle ear secretion in cases of longstanding SOM.

MATERIAL AND METHODS

Nasopharyngeal cultures in SOM. These were obtained from 76 children with SOM of more than three months duration. The diagnosis was made from the typical clinical picture including otomicroscopy and was confirmed at paracentesis under general anaesthesia. Children with recent or present upper respiratory tract infection were excluded. One culture was made from each of the children while still under general anaesthesia. Anaesthesia made it easier to avoid the anterior part of the nose at sampling and permitted the swab to stay on the surface of the adenoid for at least 10 seconds. The swab was inserted into a modified Stuart

Table I. *Bacterial findings in the nasopharynx and the middle ear secretions from children with SOM. Number of cases*

	Naso-pharynx	Middle ear
<i>Haemophilus influenzae</i>	12 (1)	7
<i>Streptococcus pneumoniae</i>	6	1
<i>Branhamella catarrhalis</i>	6 (4)	2
<i>Haemophilus influenzae</i>	16	
<i>Streptococcus pneumoniae</i>		
<i>Haemophilus influenzae</i>	2	
<i>Branhamella catarrhalis</i>		
<i>Streptococcus pneumoniae</i>	10	
<i>Branhamella catarrhalis</i>		
<i>Haemophilus influenzae</i>	7	1
<i>Streptococcus pneumoniae</i>		
<i>Branhamella catarrhalis</i>	1	
<i>Haemophilus influenzae</i>		
<i>Streptococcus pneumoniae</i>	1	
<i>Haemolytic streptococci A</i>		
Respir. pathogens total	60	11
No growth of respir. path.	16 Σ 76	50 Σ 61
Isolates of non-pathogens:		
<i>Staphylococcus epidermidis</i>	13	9
<i>Staphylococcus aureus</i>	13 (11)	1 (1)
<i>Streptococcus viridans</i>	14	1
<i>Neisseria</i> sp.	5	1
<i>Haemophilus parainfluenzae</i>	1	

() = beta-lactamase producing.

medium and transported to the laboratory within a few hours. Culture and identification of isolated pathogens were made by conventional technique.

Middle ear cultures in SOM. In a partly overlapping series, samples of middle ear effusions were obtained from 61 children with identical criteria of SOM. The specimens were aspirated at paracentesis under general anaesthesia. No attempt was made to clean the ear canals or the tympanic membranes. The secretions were transferred to sterile tubes and transported and cultured as described above.

RESULTS

Nasopharyngeal cultures in SOM. Pathogens were isolated in 60 cases (79%). *Haemophilus influenzae* and *Streptococcus pneumoniae* were the most common bacteria, each present in 38 respectively 40 cultures (50 resp. 53%).

Branhamella catarrhalis was found in 25 specimens (33%). Haemolytic streptococci group A were isolated in one case. A single pathogen was isolated in 24 cases (32%), two pathogens were found in another 28 cases (37%) and three pathogens in 8 cases (11%). See Table I.

Middle ear cultures in SOM. Pathogens were isolated in 11 cases (18%). *H. influenzae* was present in 7, *S. pneumoniae* in one and *Br. catarrhalis* in two cases, and in the last case all three pathogens in combination. See Table I. In 44 cases cultures were obtained from both nasopharynx and middle ear secretions. The results are shown in Table II. In all cases where pathogens were found in the middle ears the same pathogens were also isolated from the nasopharynx. See Table III.

Conclusion. Even several months after its onset, the condition of SOM is concomitant to a nasopharyngeal colonization of respiratory pathogens. An appreciable proportion of the middle ear effusions yielded growth of the same pathogens.

DISCUSSION

It is a well-known fact that pathogens, even *S. pneumoniae*, often survive in the nasopharynx during the course of a seemingly adequate penicillin therapy (Kamme et al., 1970; Herberts et al., 1971; Frölund Thomsen et al., 1975). The reason for this truly remarkable fact has not been found. The nasopharynx with its adenoid may be looked upon not only as a bacterial reservoir under normal conditions (Norstedt, 1967; Nylén, 1975), but also as a refuge for even the most penicillin-sensitive of pathogens during penicillin therapy. In a preliminary report very low levels of penicillin were found in the adenoid tissue (Sundén & Schiratzki, 1977).

Conventional nasopharyngeal samples are taken from the secretions on the surface of the adenoid. In recent investigations where the homogenized adenoid tissue was the subject for bacteriologic assay, all samples yielded

Table II. *Bacterial findings in 44 cases of SOM where cultures were made from both middle ear and nasopharynx*

	Number of cases where respir. pathogens were found
In middle ear and nasopharynx	8
In nasopharynx only	29
In middle ear only	0
No growth in either locality	7

growth of various species of bacteria (Raukonen et al., 1979; Brook et al., 1980; Brook, 1981a). Of special interest was the frequent finding in the latter reports of several beta-lactamase producing non-pathogenic bacteria together with e.g. *S. pneumoniae*. This finding suggests that betalactam-antibiotics are wholly or partly inactivated in the adenoid and this could be an explanation to the peculiar faculty of the nasopharynx to act as a bacterial refuge during penicillin therapy. It should be observed that there is no evidence for a bacterial invasion of the adenoid tissue itself (Lundberg & Lönnroth, 1979). In all probability the bacteria found in homogenized adenoids stem from the bottom of the crypts and furrows, almost inaccessible to ordinary nasopharyngeal swabbing.

There is some uncertainty as to which species of bacteria are pathogens in SOM. In the present investigation we have excluded the frequent occurrence of *Staphylococcus epidermidis* and of *Staph. aureus* (see Table I). These bacteria are to be looked upon as contaminants from the skin of the ear canal or the anterior part of the nose (Brook, 1981b). There is no doubt but that *H. influenzae* and *S. pneumoniae* are pathogens. *Br. catarrhalis* is isolated from pus in cases of sinusitis and acute otitis media with increasing frequency and is therefore commonly classified as pathogen. Furthermore, there is an increase in the number of beta-lactamase producing strains of *Br. catarrhalis* (Kamme, 1980). In

the present material from the nasopharynx four of 25 isolates of *Br. catarrhalis* and one of 38 cultures of *H. influenzae* were beta-lactamase producing.

In the literature the frequency of bacterial findings from middle ear effusions in SOM range from 22 to 52% (e.g. Kokko, 1974; Healy & Teele, 1977; Palva et al., 1978; Giebink et al., 1979). However, if only the respiratory pathogens discussed above are included, the frequency decreases to 9–26%. Bacteria are more often found by microscopy of stained smears than by culture (Liu et al., 1975) (Fig. 1).

This investigation lacks a control material, for obvious reasons. Normal children are not subjected to general anaesthesia, sampling can therefore not be performed in the same way. Laurell et al. (1980) gathered a control material comprising 29 somewhat older children that were subjected to general anaesthesia for non-otologic reasons and they found respiratory pathogens in 19 cases. It must be taken for granted that an appreciable proportion of nor-

Table III. *Bacterial findings in 8 cases where respiratory pathogens were found in both the middle ear effusion and the nasopharynx*

Case no.	Middle ear secretion	Nasopharynx
1	<i>H. infl.</i>	<i>H. infl.</i>
2	<i>H. infl.</i>	<i>H. infl.</i>
3	<i>H. infl.</i>	<i>H. infl.</i> <i>Br. catarrh.</i>
4	<i>H. infl.</i>	<i>H. infl.</i> <i>S. pneum.</i> <i>Br. catarrh.</i>
5	<i>S. pneum.</i>	<i>S. pneum.</i> <i>H. infl.</i>
6	<i>Br. catarrh.</i>	<i>Br. catarrh.</i> <i>S. pneum.</i>
7	<i>Br. catarrh.</i>	<i>Br. catarrh.</i> <i>H. infl.</i> <i>S. pneum.</i>
8	<i>H. infl.</i> <i>S. pneum.</i> <i>Br. catarrh.</i>	<i>H. infl.</i> <i>S. pneum.</i> <i>Br. catarrh.</i>

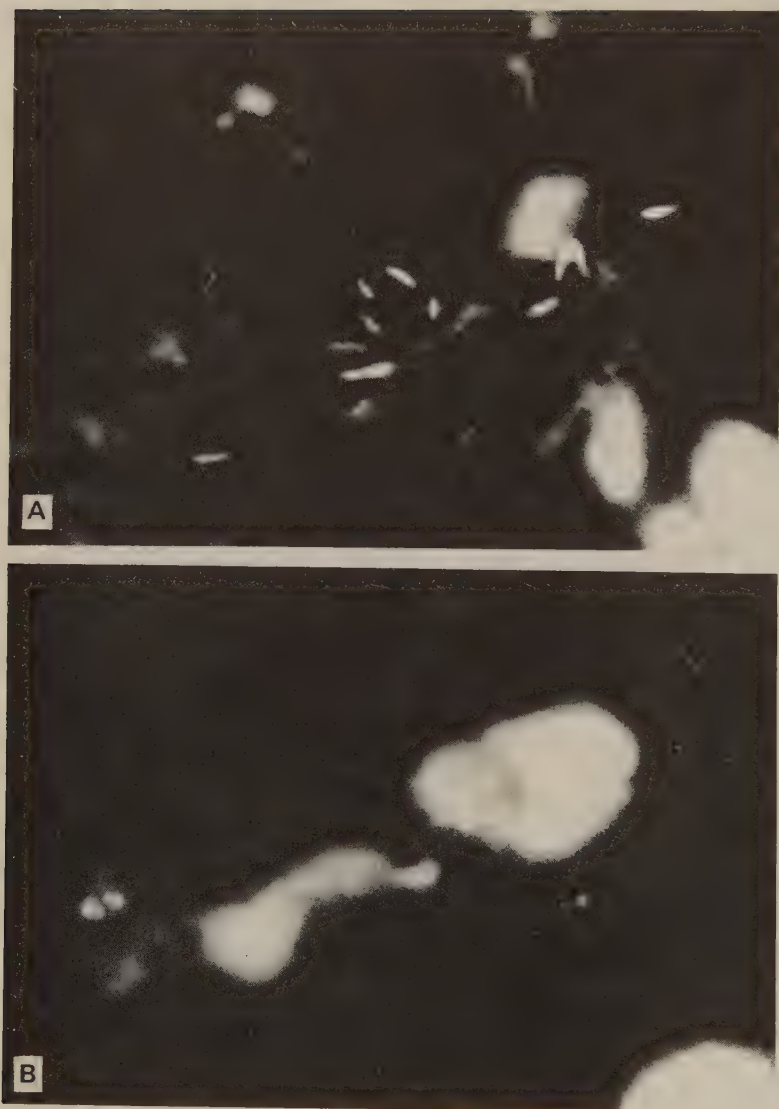


Fig. 1. (A) Bacilli and (B) cocci against a background of mucus and some cell debris. Fluorescence microscopy of smear of middle ear effusions from cases of long-standing SOM. Unfixed material, stained with acridine orange at low pH according to Kronvall & Myhre (1977). Original magnification, $\times 1010$.

mal children harbour respiratory pathogens in the nasopharynx. It is a matter of speculation whether they carry the bacteria always or for shorter or longer periods only.

In lieu of a true control material it is difficult to estimate the results of the nasopharyngeal cultures. On the face of the present evidence there is a four- to five-fold increased incidence of nasopharyngeal pathogens in SOM compared to normal children. However,

it may be safely stated that nasopharyngeal pathogens are less frequent in SOM than in acute otitis media, 84% (Ingvarsson et al., cf. Lundgren & Rundcrantz, 1976; Laurell et al., 1980) or 79% (present investigation) as opposed to nearly 100%.

It should be emphasized that in the present investigation the material is uniform. All children had a duration of SOM of more than three months, despite periodic conventional treat-

ment with decongestives. The finding of respiratory pathogens in the middle ear effusions indicates with some certainty that infection with subsequent inflammation is an etiologic factor. The occurrence of bacteria in SOM even three months after the clinically confirmed onset points to a very low virulence, if the bacteria are presumed to have been present in the secretion for the whole time.

SOM is characterized by its insidious onset. However, children with SOM frequently complain of bouts of dull earache with a duration of between half a day to a few days, at times concomitant to an upper respiratory tract infection, but just as often not (Willis, 1977). The intervals are typically symptom-free with the exception of a mild hearing loss. It seems tempting to postulate that these bouts of earache in SOM are caused by re-infections. Thus, there would be a gradual transition from children with only one short episode of SOM to those that have several periods of SOM but with disease-free intervals to others where one bout is superimposed on the previous and who have no intervals free of disease.

The nasopharynx naturally occupies a central position in this context. Respiratory pathogens carried on the surface of the adenoid could very possibly invade the middle ear, specially if the mucociliary activity of the eustachian tube is impeded by e.g. very viscous secretion. The observation that the middle ear effusions yielded the same strains of bacteria that were found in the nasopharynx (Table III) supports this line of reasoning. The nasopharyngeal colonization of respiratory pathogens, even several months after the debut of SOM, may thus be of an etiologic significance.

The good results obtained by treating SOM with grommets indicate that infection is not the only etiologic factor. There is a *circulus vitiosus*-like process in SOM where the milieu is shifted to become anaerobic in character (Sadé & Weissman, 1977), which in itself prolongs the disease. The use of grommets momentarily breaks this process. There are,

however, reports that enthusiasm over grommets induces an over-use (Mawson & Fagan, 1972; Tos & Poulsen, 1976; Brown et al. 1978). The situation is similar to that regarding acute otitis media and antibiotics. Insertion of grommets has become the most frequent surgical procedure at our ENT clinic. In a county where slightly less than 2 000 children are born each year, no less than 662 general anaesthesias were performed in 1980 on the indication of SOM.

Nevertheless, it is not surprising that grommets have gained such popularity, as there is a lack of reliable alternatives in the management of SOM. An attitude of masterly inactivity only, waiting for spontaneous resolution, is difficult to uphold if no progress is observed as the months pass, especially in those cases with obvious hearing loss. Oral decongestants were previously believed to be useful in the treatment of SOM. They have now been proven to be of no value (Edström et al., 1977; Fraser et al., 1977; Miller et al., 1977), there are even results implying that their use prolongs the disease by interfering with the mucociliary activity (Peerless & Noiman, 1980; Klein et al., 1980). Adenoidectomy has lost many of its previous indications. Some investigations have indicated that, apart from cases with nasopharyngeal occlusion, children with SOM do not benefit from adenoidectomy (Rynnel-Dagöö et al., 1978). On the other hand, it has been reported that adenoidectomy performed early in the course of SOM may reduce the number of relapses in a longer perspective (Elverland et al., 1981). Even though adenoidectomy has a long tradition as a therapeutic measure in the treatment of SOM, the dissenting opinions clearly throw doubt on its indispensability on this indication.

In view of the present findings it is tempting to use antibiotics to eradicate the respiratory pathogens in the nasopharynx and the middle ear effusions. This is not an original thought (Lundgren & Rundcrantz, 1976; Persico et al., 1978; Lim et al., 1980), penicillins being the

most widely tried agents. The results have been disappointing. However, the occurrence of beta-lactamase-producing bacteria (Brook, 1981a) indicates that a suitable antibiotic ought to be unaffected by beta-lactamase. Antibiotic treatment may have a place in the future management of SOM. Marks et al. (1981) tested co-trimoxazole and achieved resolution of SOM twice as often as with decongestants only. Erythromycin penetrates not only the adenoid tissue but also the secretions from its mucosa, more than ten times the MIC of *S. pneumoniae* (Sundberg et al., 1979; Sundberg et al., present supplement). There are, however, no clinical studies to ascertain whether the penetration of erythromycin is consistent with nasopharyngeal bacterial eradication or not. Furthermore, we do not know for how long time the nasopharynx will keep free of pathogens—reinfection or recolonization may be a matter of a short period of time only.

In clinical practice we need a method to single out those cases of SOM that really need grommets. There is no such method available today. The management of SOM still largely depends on a period of conservative measures interwoven with optimistic inactivity. Finally, a large proportion of the children are subjected to surgical procedures.

In conclusion. Evidence is accumulating showing that SOM is of infectious etiology, directly or indirectly via the nasopharynx. There is no single etiological agent—the most common bacteria are *H. influenzae*, *S. pneumoniae* and *Br. catarrhalis*, appearing as single species or in various combinations. The increasing isolation of beta-lactamase producing strains poses a therapeutic problem. There is cause to believe that an antibiotic with special characteristics should be looked for, to be used on strictly chosen indications in an attempt to increase the resolution of SOM.

ZUSAMMENFASSUNG

In Bakterienkulturen aus dem Nasopharynx von Kindern mit einem chronischen Tubenmittelohrkatarrh, ent-

nommen in Narkose, ist in 79% ein Wachstum von pathogenen Keimen der Atemwege nachgewiesen worden. Dabei hat man keine anderen Zeichen einer gerade durchgemachten Infektion im HNO-Bereich gefunden.

Bakteriologische Untersuchungen von Mittelohrsekret zeigten in 18% der Fälle Wachstum von pathogenen Bakterienstämmen. Hierbei handelt es sich um die gleichen Bakterien, die man auch im Nasopharynx fand, nämlich *Haemophilus influenzae*, *Streptococcus pneumoniae* und *Branhamella catarrhalis*, entweder als Monokultur oder in verschiedenen Kombinationen. In mehreren Fällen fand man beta-laktamasproduzierende Stämme. Auf Grund der vorliegenden Resultate muss diskutiert werden, ob die konventionelle Behandlung des Paukenergusses mit einer speziellen antibiotikatherapie kombiniert oder ersetzt werden kann.

RÉSUMÉ

Les cultures du rhino-pharynx obtenues par anesthésie générale sur des enfants atteints d'otite sécréteuse médiane (SOM) prolongée, révèlent dans 79% des cas une colonisation de microbes pathogènes respiratoires, bien qu'aucune infection récente dans la région ORL n'ait pu être découverte. Dans une série partiellement enveloppante de cultures des décharges de l'oreille médiane on a pu isoler les mêmes souches de microbes pathogènes respiratoires dans 18% des cas. Les microbes, *Haemophilus influenzae*, *Streptococcus pneumoniae* et *Branhamella catarrhalis*, apparaissent en espèces isolées ou bien en combinaisons diverses. La présence de microbes produisant du beta-lactamase peut rendre les antibiotiques betalactam inactifs. On peut présumer qu'un antibiotique à caractéristiques particulières devrait être choisi pour être utilisé sur indications strictement spécifiques, pour essayer d'augmenter la résolution des otites sécréteuses médianes.

RESUMEN

Cultivos nasofaríngeos tomados bajo anestesia general, de niños con Otitis Media Serosa (SOM), mostraron colonización de bacterias patógenas respiratorias en 79% de los casos, aunque no había evidencia de reciente O.R.L. infección. En una parte de cultivos tomados simultáneamente de la secreción del oído medio, se pudo aislar las mismas especies de bacterias patógenas que en los cultivos faríngeos, en el 18% de los casos. *H. influenzae*, *S. pneumoniae* y *Br. catarrhalis*, aparecieron como única especie o como diferentes combinaciones. La presencia de bacterias productoras de beta-lactamasa inactiva el grupo betalactam de antibióticos. Hay razón para creer que un antibiótico con características especiales debería buscarse, para utilizarlo en indicaciones estrictamente escogidas con el fin de mejorar la resolución de la SOM.

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THE EFFECT OF ERYTHROMYCIN ON THE NASOPHARYNGEAL PATHOGENS
IN CHILDREN WITH SECRETORY OTITIS MEDIA

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Abstract. Seventy-five children not older than 11 years, with secretory otitis media of more than three months duration were randomly divided into two groups prior to myringotomy. One group remained untreated, whereas the other received erythromycin ethylsuccinate (Abbotycin®) in standard dosage for the last 10 days before surgery. Nasopharyngeal cultures were taken under general anaesthesia, which ensured a uniform mode of sampling.

In the erythromycin-treated group the occurrence of *Streptococcus pneumoniae* (3%) and *Branhamella catarrhalis* (0%) was significantly lower than in the control group (35% and 32%, respectively); and the frequency of cultures with no pathogen was significantly higher in the treated group. The occurrence of *Haemophilus influenzae* remained essentially unchanged.

INTRODUCTION

Healthy children carry respiratory pathogens (*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Branhamella catarrhalis* and β -hemolytic streptococci) in the nasopharynx with a frequency varying from 18% to 63% (e.g. Nylén, 1975; Ingvarsson et al., 1982). These pathogens are recovered from the nasopharynx in almost 100% of patients with acute otitis media (Howie & Ploussard, 1971; Kamme, 1971). In Secretory Otitis Media (SOM), the rate is about 80% (Ingvarsson et al., unpublished data cited in: Lundgren & Rundcrantz, 1976; Laurell et al., 1980; Sundberg et al., 1981). In the latter study *S. pneumoniae* was found in 53%, *H. influenzae* in 50% and *B. catarrhalis* in 33% of the patients.

A remarkable feature of respiratory pathogens - even those extremely antibiotic-susceptible such as *S. pneumoniae* - is the ability to remain unscathed in the nasopharynx during and after a seemingly ample course of betalactam antibiotic therapy (Kamme et al., 1970; Herberts et al., 1971; Frölund Thomsen et al., 1975; Melén et al., 1982; Puhakka et al., 1982; Virolainen et al., 1982). Various explanations of this phenomenon have been advanced. These include the role of local colonization of betalactamase-producing bacteria (Schwartz et al., 1979; Brook, 1981), and an insufficient penetration of antibiotics to nasopharyngeal tissues and secretions (Nylén, 1975).

Erythromycin has been shown to penetrate both the adenoid tissue and respiratory tract secretion in SOM, reaching levels well above the MICs of the respiratory pathogens, with the exception of many strains of *H. influenzae* (Sundberg et al., 1979, 1981).

The aim of the present investigation was to study the effect of erythromycin on the nasopharyngeal pathogens in children with long-standing SOM.

MATERIAL AND METHOD

The study includes 75 children between 1½ and 11 years of age with SOM on one or both sides for more than three months. The criterion of SOM was a non-purulent effusion behind an intact tympanic membrane at otomicroscopy and a type "B" curve at tympanometry. When the children had been followed at intervals of six weeks for three months or more without resolution of SOM, surgery (myringotomy) was recommended, to be performed under general anaesthesia.

At this stage every second consecutive child in the care of each otologist separately was allotted to the test group. This group was given erythromycin ethylsuccinate (Abbotycin®) orally 20-30 mg/kg body weight twice daily during the last 10 days before surgery. The last dose was administered not more than 15 hours before surgery. Informed consent was obtained from the parents. The control group was given no treatment before surgery.

The test group consisted of 38 and the control group of 37 cases. The two groups were similar in sex and age distribution (Table I). Children with recent (within four weeks) or present respiratory tract infection at operation were excluded.

Table I. *Sex and age distribution*

Patients	n: total	n: males	n: <4 years	age: mean	age: S.D.
Control group	37	20	8	6.0	2.7
Treated group	38	20	11	6.6	2.8

One nasopharyngeal culture was taken from each child under general anaesthesia. This made it easier to avoid the anterior part of the nose and permitted the swab to be gently pressed on the surface of the adenoid for at least 10 seconds. The swab was inserted into a modified Stuart medium and transported to

the laboratory within a few hours. Culture and identification of isolated pathogens were made by conventional techniques. Bacterial growth could be roughly quantified as abundant, moderate or sparse by estimating colony numbers on the plate where the primary inoculum was spread with a wire-loop by a standardized method. Sensitivity testing was performed by disc diffusion technique.

The occurrence of possible differences between the two groups was tested by χ^2 analysis with Yates' correction.

RESULTS

Details are supplied in Tables II and III.

Table II. *A survey of the results*

Pathogens	Control group	Treated group	χ^2 value	Level of significance
No pathogen	5	20	11.384	$p < 0.001$
One pathogen	15	18	0.150	$p > 0.5$
Two or more pathogens	17	0	20.296	$p < 0.001$
<i>S. pneumoniae</i>	13	1	11.202	$p < 0.001$
<i>B. catarrhalis</i>	12	0	12.595	$p < 0.001$
<i>H. influenzae</i>	25	17	3.181	$p > 0.5$
β -hemolytic streptococci	2	0		

Table III. *Haemophilus influenzae* strains isolated. Quantitative assessments in the primary cultures and results of sensitivity testing to erythromycin. Number of strains.

	Control group	Treated group
Growth abundant	15	4
moderate	6	5
sparse	4	8
Sensitive	0	2
Intermediate sensitivity	24	11
Resistant	1	4
Total	25	17

The following species were regarded as nasopharyngeal pathogens: *S. pneumoniae*, *H. influenzae*, *B. catarrhalis* and β -hemolytic streptococci group A, C and G. These usually occurred alone or in various combinations. In many cases there was growth of other bacteria: *Staphylococcus aureus*, *S. epidermidis*, α -hemolytic streptococci, *Corynebacterium* spp, *H. parainfluenzae* or *Neisseria* spp including one culture with *N. meningitidis*. In three cases there was no growth at all in the culture.

Respiratory pathogens were found in 32/37 children in the control group (86%). Two or more pathogens were recovered in 17 cases. A total of 53 strains were isolated: 25 *H. influenzae*, 13 *S. pneumoniae*, 12 *B. catarrhalis* and 2 β -hemolytic streptococci (one group A and one group G).

The nasopharyngeal cultures in the erythromycin-treated group differed from the control group in the following respects:

The total isolation rate of pathogens was significantly lower (47% vs 86%, $p < 0.001$). The same applied to the finding of two or more pathogens in the same sample (0% vs 46%, $p < 0.001$).

The frequency of *S. pneumoniae* (3% vs 35%, $p < 0.001$) and *B. catarrhalis* (0% vs 32%, $p < 0.001$) was significantly lower.

The occurrence of *H. influenzae* was not significantly lower in the erythromycin-treated group (45% vs 68%, $p > 0.05$). When the isolates were quantitatively assessed, there was no significant difference in the growth at culture of these strains ($p > 0.05$). The sensitivity to erythromycin of the *H. influenzae* strains was similar in both groups.

β -hemolytic streptococci were not found in the erythromycin-treated group.

DISCUSSION

The results in the control group confirm previous observations, viz. that 80-85% of untreated children with SOM harbour respiratory pathogens in the nasopharynx (Ingvarsson et al., unpublished data cited in: Lundgren & Rundcrantz, 1976; Laurell et al., 1980). *H. influenzae* was more common in the control group than *S. pneumoniae*. Nevertheless, the present distribution of pathogens did not differ significantly from the usual pattern in SOM cases of more than three months duration (Sundberg et al., 1981).

The MICs of *S. pneumoniae* and *B. catarrhalis* for erythromycin are below 0.1 mg/l, with very few exceptions (Kamme, 1970; Dette, 1979), and the recovery of both these bacteria was significantly lower in the erythromycin-treated group compared to the untreated group. On the other hand, *H. influenzae* was found in the same frequency in both the treated and the untreated group. The MICs of most strains of *H. influenzae* are above 1.0 mg/l for erythromycin (Kalm et al., 1975). The results of the present investigation thus indicate that the local erythromycin level must have been between these two MIC values: 0.1 and 1.0 mg/l.

Nasopharyngeal pathogens have not been observed in the adenoid tissue itself, but have been localized to the secretions of the respiratory mucosa that covers the adenoid surface down into every furrow (Lundberg & Lönnroth, 1979). A local drug activity must therefore be present in the secretion in order to influence the respiratory pathogens.

In blood-free homogenates of adenoid tissue, including the secretion covering its mucosa, the concentration of erythromycin followed the concomitant plasma levels closely (Sundberg et al., 1981). Three hours after each dose the levels were in the range 1.0 - 1.5 mg/l in plasma and adenoid tissue homogenate. It is well known that the concentration of an anti-

biotic is generally lower in the secretion than in the tissue (Eneroth & Lundberg, 1976). In children with SOM a middle ear effusion level of erythromycin, using a similar dosage, was 1.0 mg/l (Sundberg et al., 1979). By analogy it can be inferred that the nasopharyngeal secretions obtain levels of the same magnitude, i.e. about 1.0 mg/l. This level is in the order of 10 times the MICs of *S. pneumoniae* and *B. catarrhalis* and β -hemolytic streptococci, but less than the MICs of most strains of *H. influenzae*.

Only two strains of *H. influenzae* from the 42 strains isolated in both groups were sensitive to erythromycin, 35 showed intermediate sensitivity and 5 were resistant. It was thus not surprising that there were no indications of an antibacterial effect on the *H. influenzae*. The erythromycin sensitivity pattern of the present *H. influenzae* strains differed markedly from the more sensitive strains found in clinical isolates from outpatients (Forsgren & Walder, 1982).

Nasopharyngeal sampling may pose some problems in unanaesthetized and sometimes uncooperative children, and some loss of data is likely to occur. During general anaesthesia the conditions are close to ideal for nasopharyngeal sampling. The results of this study, including the finding of no growth of pathogen, can therefore be regarded as highly reliable.

It should be observed that there was no difference between the two groups of children. The age and sex distribution was the same. There are reports that the relative nasopharyngeal carrier rate of *S. pneumoniae* is highest below the age of four (e.g. Lundgren, 1972; Schwartz et al., 1977) - the proportion of children below this age was the same in both groups. The clinical selection - cases with SOM for more than three months in otherwise uninfected children - ensured a high degree of uniformity.

In conclusion: in children with persistent SOM, erythromycin given in standard dosage for 10 days resulted in a significantly lower frequency of *S. pneumoniae* and *B. catarrhalis* in the

nasopharynx, compared to untreated children. The occurrence of *H. influenzae* remained essentially unchanged.

The results may be explained by local drug activity, in accordance with previous studies on the penetration of erythromycin to secretions of respiratory mucosa and to adenoid tissue (Sundberg et al., 1979, 1981).

ZUSAMMENFASSUNG

75 Kinder im Alter bis zu 11 Jahren mit einer mehr als dreimonatigen Anamnese für einen Tubenmittelohrkatarrh wurden nach Randomisierung in zwei Gruppen aufgeteilt und der Parazentese in Vollnarkose zugeführt. Die eine Gruppe erhielt keine Behandlung, während die andere mit Erythromycin-Ethylsuccinat (Abbotycin®) in Standarddosierung während der letzten 10 Tage vor dem Eingriff behandelt wurde. Bakterienkulturen vom Nasopharynx wurden gleichzeitig und während der Vollnarkose entnommen, womit eine einheitliche Entnahmetechnik erreicht wurde.

In der erythromycinbehandelten Gruppe fand man signifikant weniger *Streptococcus pneumoniae* (3%) und *Branhamella catarrhalis* (0%), jedoch in der Kontrollgruppe 35% respektive 32%. Überhaupt war die Anzahl der Kulturen ohne Wachstum pathogener Bakterien signifikant höher in der behandelten Gruppe. Das Wachstum von *Haemophilus influenzae* blieb in beiden Gruppen unbeeinflusst.

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